TOLERANCE IN *COLLETOTRICHUM COFFEANUM* NOACK TO BENOMYL AND OTHER RELATED SYSTEMIC FUNGICIDES

By

Agnes Wakesho Kirangu
B.Sc. Agriculture (University of Nairobi)

Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in University of Nairobi, Kenya.

April 1983

To Kirangu and Mkakisha

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

A. Kiranga.

9th June 1983

This thesis has been submitted for examination with our approval as University Supervisors.

DR. D.M. MUKUNYA

Daniel Mukunya

10th June, 1983

DR. E.M. GATHURU

E. Gathuru

10th June, 1983

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisors Dr D.M. Mukunya and Dr E.M. Gathuru of the Department of Crop Science, Faculty of Agriculture, University of Nairobi, for inspiration, encouragement and guidance throughout the period of the work.

I am also indebted to Mr D.J. Walyaro of Coffee Research Foundation, Ruiru, for guidance in statistical analysis of the data.

I am grateful to the Coffee Research Foundation, Ruiru, for allowing me to use the laboratory facilities.

I wish to thank the Ministry of Agriculture, Kenya and Coffee Research Foundation through the Director of Research, Coffee Research Foundation, Ruiru, for financial support.

I would like to thank Mr Kit Karanja of Coffee Research Foundation, Ruiru, for technical assistance in photography. Finally, I would like to thank Mr Morris Mileso and Miss Kellen Kariuki of Coffee Research Foundation, Ruiru, for technical assistance and Miss Madrine Rugono for typing the thesis.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABSTRACT

Two benomyl tolerant strains of Colletotrichum coffeanum Noack were isolated from infected green berries from Migaa and Kibubuti estates in Kiambu District. The estates were known to have a history of benomyl coffee berry disease (CBD) control programme. The sensitive strain was isolated from a plot that had never been sprayed with fungicides at Coffee Research Station, Ruiru, in Kiambu District.

An account is given of the mycelial growth, pigmentation and sporulation of benomyl tolerant and sensitive strains grown under similar conditions on synthetic media. Slight differences were observed in pigmentation, sporulation, zonation and radial growth of mycelia.

It was noted that conidia from the benomyl tolerant strains showed a slight delay in germination compared to those of the sensitive strain. In all cases spore suspensions containing lower numbers of conidia gave lower levels of germination and took longer time to germinate compared with the spore suspensions containing higher numbers of conidia. Conidia washed several times after harvesting from malt extract agar (MEA) also gave lower level of germination compared to the unwashed conidia and washed conidia in glucose solutions. Higher doses of benomyl lowered percent germination and also delayed germination of conidia.

In vitro mycelial growth of benomyl tolerant strains was less inhibited on MEA containing 1000 µg/ml benomyl than that of a sensitive strain on MEA with 1.0 µg/ml benomyl. Differences in sporulation were found among all strains in in vitro. No reduction of conidial size was observed. Conidia of tolerant strains, harvested from MEA amended with different concentrations of benomyl, did not lose their viability and were able to germinate in distilled sterile water. All strains in culture produced colour variants by sectoring.

Tolerance was retained for at least six months after repeated subculturing on fungicide free MEA.

Benomyl tolerant strains were also tolerant to other benzimidazole fungicides (Derosal, Bavistin). *In vitro* mycelial growth and sporulation of the tolerant strains were less inhibited on MEA containing 1000 µg/ml a.i. Derosal or Bavistin than that on MEA with 1000 µg/ml benomyl.

Tolerant strains were found to be very competitive and readily produced anastomosing germ tubes and hyphae. They were found pathogenic and produced typical CBD symptoms in the field and transmitted the benomyl tolerance to the new conidia. Benomyl application tended to reduce percent berry infection especially where the sensitive strain was used for berry inoculation.

Captafol and Copper 50% formulation alone or in combination with benomyl were effective in controlling all strains of *C. coffeanum* *in vitro*. Benomyl at very low concentrations (1.0 µg/ml) was very effective in controlling the benomyl sensitive strain *in vitro*.

1. INTRODUCTION

Arabica coffee is the leading foreign exchange earner in Kenya. It accounts for 30 - 40% of the total domestic exports with an average annual value of K£ 115 million over the years 1978 - 1980. Approximately 95% of coffee grown in Kenya is exported (Anon., 1979/7 & 1979/80). There are about 265,000 small scale farmers, who are organized in about 153 co-operative societies which own 568 factories and there are 740 large coffee estates (van der Vossen and Walyaro, 1981). Together with workers who find permanent or seasonal employment on the large estates, it is estimated that more than 10% of the population in Kenya depend on coffee for their livelihood. Coffee berry disease and to a lesser extent coffee rust are the major constraints to economic production of arabica coffee in Kenya.

1.1. Coffee berry disease and *Colletotrichum coffeanum* Noack

According to Nutman and Roberts (1960 a) the strain of *Colletotrichum coffeanum* Noack (Sensu Hindorf) causing coffee berry disease (CBD) could have arisen by mutation from a mildly parasitic form (for example that causing brown blight) some time prior to 1922 in Western Kenya. Robinson (1974), however, proposed that the CBD strain was originally a harmless parasite of one of the diploid progenitors of *Coffea arabica* L., which occurred in the Congo and Lake Victoria basins, but was able to cause a virulent disease of cultivated *C. arabica* when this crop was introduced to that area some 80 years ago. Robinson, further suggested that resitance to CBD, which was presumably present when *C. arabica* first originated, was lost by genetic erosion during diversification in Ethiopia and during the further reduction of the genetic base of cultivated arabica coffee which occurred as selected material was taken from one coffee growing country to another. Thus, the cultivated varieties of *C. arabica* were quite susceptible to CBD when they returned to an area close to their centre of origin and once again encountered the CBD fungus.

On first appearance in Kenya, CBD caused 75% crop loss in some cases (McDonald, 1926). It caused the abandonment of coffee growing in several districts of Kenya (Bock, 1970) and Ethiopia (Robinson, 1974). It was also a factor preventing increased cultivation of arabica coffee in Angola (Da Ponte, 1966). In assessing world losses caused by coffee diseases, Crammer (1967) cautiously estimated that losses in Africa amount to 20% of the potential crop. In Kenya, Nutman (1966) estimated that in 1964 CBD alone caused a financial loss of 9.8 million U.S. dollars equivalent to the value of just under 10,000 tons or a loss of 18.9% from the potential crop. In 1967/68 a severe CBD epidemic resulted in overall crop loss of between 25 and 30%. On individual farms, overall crop loss due to CBD fungus could be as high as 80% or more (Anon, 1976).

By 1952 it was known that some degree of control of CBD could be exercised by fungicide sprays (Rayner, 1952) but little real success had been achieved. Losses experienced on individual crops have depended both on the severity of the disease and the yield potential of the various varieties under the prevalent conditions. However, in all places where the disease has been present and conditions favourable to its development, control by an annual fungicide spray programme doubled or trebled yields (Griffiths et al., 1971). In one experiment in Kenya in 1968 (Vine et al., 1973 a) unsprayed coffee yielded 276 kg/ha which was less than one fifth of the 1522 kg/ha produced from plants in which the disease was well controlled. This indicates the losses which a severe epidemic of the disease can cause.

A large array of fungicides have been recommended for CBD control. Their chemical nature is quite varied. Benomyl proved to be very effective and was thus widely used on coffee. As benomyl was extensively used on coffee for CBD control with consequent outbreaks of tolerance to benomyl in some coffee estates, there has been concern that tolerance might develop and spread in other coffee plantations. Thus, this study was undertaken to provide more information on the characteristics of the tolerant strains as compared to the sensitive strain of C. coffeanum.

This study reports the isolation of benomyl tolerant strains of C. coffeanum. It provides information on spore germination, sporulation, growth in the presence of benomyl, stability of tolerance, existence of cross-tolerance to other benzimidazole fungicides, possible mode of inheritance to tolerance, strain ability to compete in mixed populations, their virulence, the extent to which tolerant strains survive in the absence of benzimidazole fungicides and their in vitro control.

2. LITERATURE REVIEW

2.1. Introduction:

Coffee berry disease (CBD) caused by the fungus C. coffeanum Noack is a serious disease of Coffea arabica L. in Kenya. C. coffeanum attacks flowers, green berries, ripe berries and sometimes leaves of young suckers. The classification of CBD was based on the growth of the berries and the type of plant parts attacked. CBD affects immature coffee berries. The susceptibility of the young berries increases as they expand during the period of 4 - 16 weeks after flowering (Mulinge, 1970). "Brown blight" is a term used when the pathogen attacks ripe coffee berries. Berry "scab" is a term used when the development of the active lesions, at any stage of the berry, is halted by the onset of dry conditions and the berry wall may be the only part affected. When the disease attacks leaves, it is referred to as "leaf anthracnose" and if on branches as anthracnose of the branches.

2.2. History and geographical distribution of coffee berry disease:

After its initial detection in Western Kenya close to the Ugandan border (McDonald, 1926), the disease spread southwards and eastwards infecting all coffee areas of west of the Rift Valley by the late 1930's (Royner, 1952). Since 1950, it has spread to all coffee areas east of the Rift Valley (Firman and Walter, 1977). In the late 1960s the disease appeared in the coffee areas on the southern slopes of Mt. Kilimanjaro in Tanzania and in 1971 the disease was reported in Ethiopia (Mulinge, 1973). CBD has also been present for sometime in Zaire and Cameroon (Firman and Waller, 1977). The disease has now spread to most African countries but has not yet been reported outside Africa. However, a coffee berry necrosis that was first observed in Guatemala in 1963 is present in Costa Rica (Schieber et al., 1970) and may be similar. Agnihothrudu (1972) suggested that it may be present in India, but Hindorf and Muthappa (1974) did not find the virulent CBD strain of C. coffeanum there.

2.2.1. Economic importance:

"Leaf anthracnose" is seldom important except occasionally in nurseries. Anthracnose of the branches leading to dieback can be locally serious on certain cultivars and especially under adverse environmental conditions. The type of severe dieback specifically atrributable to C. coffeanum as reported in Central African Republic (Saccas & Charpentier, 1969) is not common elsewhere. Losses from flower infection are usually slight. "Brown blight" of ripe berries is common wherever coffee is grown but is only troublesome in areas where coffee is wet processed. In these areas severe outbreaks during wet weather can cause pulping difficulties, bean staining and loss of quality (Hocking, 1966). Great losses occur where CBD is present. Boison (1960) and Saccas & Charpentier (1969) reviewed many reports of severe losses from the disease and losses of up to 80% of the crop were not unusual in some plantations.

2.3. Symptomatology:

Boison (1960) distinguished two groups of symptoms: anthracnose of leaves, fruits and branches, characterised by lesions on the different organs and dieback characterised by desiccation of the extremities of the branches. The various symptoms have been described in detail by several workers. The fungus can also be present as latent infection.

2.3.1. Anthracnose of leaves:

Necrotic brown lesions are produced usually on the margins of leaves and as they become older they may develop concentric rings on which the acervuli are visible as small black dots mostly on the upper surface of the leaf. Early reports of Butler (1918) and Small (1926) recorded such symptoms as being fairly common in India and Uganda respectively, and they have been subsequently reported from most coffee growing countries. Although the fungus can be a primary pathogen of leaves (Hocking, 1966) it

also occurs in leaf lesions following infection by such fungi as Hemileia vastratrix Berk & Br. (Butler, 1918) and Cercospora coffeicola Berk & Cooke (Alandia & Bell, 1957) or following injury from nutrient deficiencies (Wellman, 1961) or insects (Small, 1926). The preselection test applied when the seedlings are 8-10 months old, takes advantage of the susceptibility of shoot tips (van der Vossen et al., 1976).

2.3.2. Anthracnose of fruits:

On ripe berries infection usually occurs as sunken patches which spread rapidly and eventually cover the whole berry (Anon., 1976). In East Africa this symptom is called "brown blight" and is the same as that described in India by Butler (1918). It has been reported in most coffee growing countries. A disease of immature fruit known locally as coffee berry disease (CBD) was first reported from Kenya in 1922 (McDonald, 1926), where it occurred in the western region at altitudes between 1800 and 2100m. Flowers are very susceptible at all stages from the pale green unopened spike. On flower petals, a dark brown streak is the first symptom. The whole flower is sometimes destroyed. The young berries are most susceptible during their expansion phase which occurs from 4 to 16 weeks after flowering (Mulinge, 1970). The early "pin head" berries are more resistant and mature green berries are again fairly resistant but become susceptible as they begin to ripen. Abscission of young infected fruit, which may occur either at the bottom or top of the fruit stalk (Rayner, 1952) occurs freely; older berries may remain attached and become black and mummified. On the green berries, infection may take two forms (McDonald, 1932; Bock, 1956). Active lesions start as small dark sunken patches which spread rapidly to involve the whole berry. The fungus sporulates freely on them and a pale pink crust of spores may be seen. In "scab" lesions, on the other hand, the lesion develops slowly and is buff coloured with scattered dark stromata; the growth of the fungus is limited and it sporulates either sparsely or not at all. In such lesions the fungus may die out and the affected area may be sloughed off,

although sometimes the fungus penetrates deeper to destroy the beans; on ripening berry scab lesions can become active. Invasion of berries by mycelial growth from the stem is of local importance only and is not a general phenomenon (Nutman & Roberts, 1960 b), but when it does happen spectacular losses of crop can occur (Nutman & Roberts, 1961).

2.3.3. Latent infections:

In isolations made from coffee tissue, Rayner (1948) found that practically every piece of tissue (healthy leaf, green berry, stem, pedicel) down to 1 mm^2 pieces of leaf produced fungal growth. These fungi consisted of Colletotrichum in a variety of forms, and species of Phoma and Phomopsis. He suggested that the fact that these fungi were present as latent infections on almost all healthy coffee tissue could explain the consistency with which they fructify on moribund or necrotic tissues and why physiologic diseases have been attributed to them. There was evidence to suggest that premature shedding of leaves; irregular bark ripening and uneven ripening of berries could be associated with the development of latent infections. Latent infection of fruit by Colletotrichum is common on many crops and has been studied by Simmonds (1963) with special reference to the banana. These infections usually become active when the fruit ripens. A feature of Colletotrichum spores is that they develop thick walled resistant appressoria when they germinate and these may survive to produce progressive infections at a later date (Meredith, 1964). Latent infections of coffee berries may turn into the brown phase as the berries ripen (Hocking, 1966). The frequency with which Colletotrichum infections develop on surface sterilized detached green berries in Kenya, especially at the stalk end, suggest that latent infections or resistant dormant appressoria are fairly abundant on young berries (Firman and Waller, 1977)

2.4. Host range:

A few workers have conducted studies which dealt directly with

cross inoculations of Colletotrichum isolates from coffee. Small (1926) isolated fungi, from many different cultivated plants in Uganda, that he considered to be identical with C. coffeanum. Although the results were erratic it was shown that under some circumstances isolates from coffee could infect other hosts and vice versa. Hocking (1971) inoculated fruits of 19 plants from 14 different families with a virulent CBD isolate from green coffee berries and presumably a different strain from coffee twigs. The CBD isolates infected the fruits of only three other species whereas the twig isolate produced active lesions on 14 of the 19 species. He suggested, therefore, that the specialized CBD strain did not have the polyphagous ability of what he called the generalized wild type races. Hindorf (1974) tested Colletotrichum isolates from 28 host plants in Kenya for pathogenicity to coffee, but found that none resembled the virulent CBD strain or produced CBD symptoms on coffee and that alternative hosts did not play a part in CBD epidemiology.

2.5. Etiology:

2.5.1. Classification of the fungus:

The fungus causing coffee berry disease, falls under the class of Fungi Imperfecti (Deuteromycetes), in the order of Melanconiales, family Melanconiaceae, Section Hyalosporae and genus Colletotrichum (Clement & Shear, 1957; Alexopoulos, 1962). There are several distinct species of Colletotrichum. In a revision of the fungi classified in the genera Colletotrichum and Gloeosporium, von Arx (1957, 1970) had recognised 13 distinct species of Colletotrichum and proposed the conservation of the genus on practical grounds despite the priority of Vermularia. Many of the fungi formerly described as Gloeosporium (a genus for which the type is actually a Marssonia sp.) were transferred to Colletotrichum. Among them was Gloeosporium coffeanum (Delacroix, 1897, as cited by Firman and Waller, 1977), the name under which this fungus was first described on coffee although

it has been more usually referred to as Colletotrichum coffeanum Noack (Noack, 1901, as cited by Firman and Waller, 1977) in coffee literature. It is a conidial state of Glomerela cingulata (Stonem) Spauld & Schrenk, and as such is equated by von Arx (1957) with Colletotrichum gloeosporioides Penz. the specific epithet having been selected from over 600 names given to the conidial state of G. cingulata. Wheller (1954) remarked that G. cingulata is a notoriously variable species and that although this is a major disadvantage for studies of the nature of parasitism it makes it well suited for basic studies of the nature of variability of fungi.

Wellman (1961) was convinced that the imperfect state occurring on coffee could be classified under both Colletotrichum and Gloeosporium because some isolates produced setae and others did not. Butler (1918) noted that setae may be absent especially in young acervuli on the leaves whereas on berries they were well developed. Setae are most commonly produced on a dry or drying substrate both in nature and artificial culture (Hendrickx, 1943 as cited by Firman and Waller, 1977; Small, 1926) and Frost (1964) had suggested that the effect of humidity on the formation of setae may be of widespread occurrence in the genus Colletotrichum.

There is great morphological variability among isolates of G. cingulata from coffee, a situation similar to that reported on other crops such as apple (Andes and Keitt, 1950), banana (Kaiser and Lukezic, 1966) and citrus (Burger, 1921, as cited by Firman and Waller, 1977). Most people working with Colletotrichum on coffee have referred to the fungus as C. coffeanum, although distinct forms or strains have been recognised by pathologists, particularly with regard to work on coffee berry disease. These are distinguishable by colony characters when grown on agar and sometimes by conidial dimensions when these are outside the normal range for C. gloeosporioides. Meiffren (1957) (cited by Firman and Waller, 1977), for example, recognised three strains based on conidial size, in the Ivory Coast, one having particularly large conidia (28 - 34.4x8 - 12.8μ)

and very long setae. The formation of conidia directly on the hyphae rather than in acervuli is a feature of certain strains when grown on agar.

Hindorf (1970, 1973 a, b) made morphological studies of Colletotrichum isolates from coffee in Kenya and discussed them in relation to the taxonomic work of von Arx (1957, 1970). In most cases the isolates proved to be the conidial states of G. cingulata but Colletotrichum acutatum Simmonds was also common. He preferred to retain the name C. coffeanum for the fungus causing CBD which was pathogenic to green coffee berries, and referred to most other isolates as C. gloeosporioides. However, the C. coffeanum described by Noack (1901) from Brazil was not a pathogen of green berries but the name has been used for practically all isolates from coffee, whether parasitic or saprophytic for over 80 years. Various authors have recognised this anomaly, such as Rayner (1941), who proposed the name C. coffeanum var. virulans for CBD pathogen, and Nutman and Roberts, who referred to it as a form of C. coffeanum. The original fungus to which Noack gave the name C. coffeanum was most probably C. gloeosporioides. C. coffeanum as originally described would therefore seem to be a synonym of C. gloeosporioides (a conidial state of G. cingulata) and thus may be taxonomically invalid for describing the CBD fungus.

2.5.2. Studies of the pathogen:

2.5.2.1. Cultural studies:

Several different media have been used in the course of investigation of the CBD fungus. McDonald (1926) tried several media in his work of identification of CBD fungus. The chief media used were coffee bean extract agar, carrot agar and coffee wood blocks. He found that the brown blight fungus from coffee berries did not reach a perithecial stage nor did that of the CBD Colletotrichum. The colour

of the mycelium of the CBD fungus always differed markedly from the rest in that the fungus invariably became coloured after a few days' growth. This colouration differed with the medium but was always consistent with each particular medium. The change from white to a dark colour appeared to coincide with the formation of the first conidia. He found that on carrot agar the first change was to green, and, with the growth in diameter of the mycelial colony, there was a gradual enlargement of the green area from the centre outwards, always leaving a narrow white periphery as long as active growth was taking place. With age the green darkened until eventually the culture was almost black and of a uniform tint. He found that the colour change on coffee wood blocks was through dark green to practically black with a white margin while active growth lasted. On coffee bean extract agar changes were not so distinct owing to the dark colour of the medium, but various shades of grey preceded the eventual blackish colour. He, also, found that the CBD fungus showed a great tendency to form dark brown chlamydospores as the medium dried out. The chlamydospores were very similar in appearance to the appressoria produced on germination of the spores and suggested that they were probably largely the cause of the very dark colour of the culture. Gibbs (1969) used 2% (Oxoid) MEA, containing 0.02% streptomycin, in the studies of isolation and characterisation of Colletotrichum spp. from coffee berries and bark. He found that the CBD (var. virulans) fungus was slow growing, profuse greyish-black aerial mycelium with conidia borne directly on hyphae. Cook (1971, unpublished) used a basal medium of Mg SO4. $7H_2O$, KH_2PO_4, peptone, agar in distilled water containing a range of glucose concentrations with or without leaf decoctions. He found that maximum spore production was after 8 days of incubation at 22°C and that the addition of leaf decoction did not stimulate sporulation.

The CBD form is present only in small amounts and never accounts for more than 5% of the total number of spores produced by sporulating bark (Gibbs, 1969). There are no records of the perfect state of the CBD

form having been produced in culture despite many attempts in Kenya, neither has the perfect state been associated with CBD infected berries in Kenya. However, Hocking et al., (1967) in Tanzania reported that perithecia were formed on green berries inoculated with the CBD form and that, after five weeks incubation, 13% of the field collected infected green berries had perithecia. A few berries with perithecia were also found on trees. In Kenya 90% of mono-ascospore cultures by Vermeulen (1970 b) from G. cingulata perithecia on coffee branches were of the ccm and 5% of the cca form. A few isolates corresponded to the two types described by Hindorf (1970), one producing mycelium bearing only perithecia and no conidia and the other, which was very rare, being the greenish mycelial form of C. gloeosporioides. Vermeulen (1970) found no evidence in Kenya that ascospores from perithecia on bark or grown in culture could infect wounded or unwounded berries.

2.5.2.2. Factors affecting conidial germination and infection:

Much of this work was done by Bock (1956) and Nutman and Roberts (1960 b), although subsequent work by several authors has increased the understanding of conditions favourable or inhibitory to conidial germination.

2.5.2.2.1. Temperature:

Nutman and Roberts (1960 b) reported that the optimum temperature for the germination of C. coffeanum conidia in water was about 22°C, but the presence of nutrients resulted in a higher optimum about 27°C although the optimum for subsequent lesion formation after inoculation remained at 22°C. The maximum temperature for germination was about 30°C (up to 35°C with nutrients) and the minimum was 15°C. However, Bock (1956) had previously shown that some infection could occur at temperatures down to 10°C. Subsequently others (Waller, 1971; Cook, 1975 b) also suggested that in the field some infection took place at

temperatures below 15°C, and Steiner (1973) (cited by Firman and Waller, 1977) confirmed Bock's findings from both laboratory and field studies.

2.5.2.2.2. Time:

At optimum temperatures, conidial germination and appresorial formation could occur within 4 hr., but at the other temperatures, germination to the stage of appresorial formation took much longer, especially at higher temperatures, where long germ tubes were formed. In an experiment in which inoculum droplets on berries were dried at interval to kill ungerminated conidia before being replaced in humid conditions to allow further disease development, Nutman and Roberts (1960 a) showed that some infection was possible in less than 3 hr. and that the number of lesions formed increased in a linear manner for infection periods of up to 20 hr.

2.5.2.2.3. Moisture:

According to Nutman (1970) germination could occur only in the presence of liquid water although earlier work by Nutman and Roberts (1960 b) had indicated that germination and infection could take place under high humidity. Meteorological data from within coffee trees (Kirkpatrick, 1935) showed that at temperatures allowing germination and infection, a saturated atmosphere in the absence of free water would be rare. Nutman and Roberts (1960 b) also studied the effect of partial desiccation on the conidia by exposing them to sunlight and by night to the dry conditions on a laboratory bench. One day's exposure to desiccation of the extruded mass of conidia on the lesions increased their infectivity markedly but subsequent drying resulted in progressive reduction of infectivity. The conidia lost infective ability totally after 10 - 12 days. On coffee berries with the more humid and shaded conditions in a coffee plantation, desiccation would be much less so that infectivity would be expected to be maintained for a longer period.

2.5.2.2.5. Altitude:

Nutman and Roberts (1960 b) considered that the relationships of CBD development with altitude, rainfall, shade and topography were all readily explicable by the experimental facts concerning conidial production, germination, growth and penetration in relation to time, temperature and humidity. It was thought that standard meteorological data could be used to predict the probable incidence of the disease in any given area. In particular they noted that widely divergent views on the effect of shade on disease incidence had been expressed (Anon., 1934; Rayner, 1952) and concluded that shade could either increase or decrease the disease depending on the altitude at which observations were made. It is widely reported not only in Kenya, but also in Angola (Da Ponte, 1966), Cameroon (Muller, 1964, cited by Firman & Waller, 1977), Tanzania (Tapley, 1964) and Uganda (Butt & Butters, 1966) that CBD is more serious at high altitudes.

During the time of the work of Nutman and Roberts, the disease was apparently restricted to altitudes above 1700 m in Kenya. They suggested (1961) that this was due to certain limits with respect to temperature and humidity for colonisation of the twigs by the CBD species. Nutman and Roberts (1969 d) attempted to explain the spread of CBD to lower altitudes during the 1960s (outbreaks now occur down to 1500 m in wet years) and its greater severity elsewhere when compared to the situation in the late 1950s. They suggested that there had been a significant climatic change during the early 1960s which might have allowed the CBD species to colonise twigs more readily at lower altitudes. This view was challenged by Griffiths and Waller (1971) who found no significant differences in temperature or rainfall between the 1950s and 1960s when they examined in detail data from 20 sites spread over both high and low altitude coffee growing areas. It was apparent, however, that variation in the rainfall distribution between years could substantially alter the cropping season. Because overlapping crops had

a significant effect on both inoculum level and CBD incidence, those factors which influenced the flowering of coffee could have an effect on disease. They showed that variation in rainfall distribution could give rise to substantial changes in cropping patterns resulting in a shift of two months or more of flowering times between different years. They also showed that there had been a substantial increase in double cropping (a subsidiary, short rains crop usually flowering in October, overlapping with a main long rains crop usually flowering in March) during the late 1950s. Nutman and Roberts (1969 b) suggested that this could be the result of CBD stimulating out of season flowering by the destruction of flowers and young berries. Griffiths and Waller (1971), however, showed that it was correlated with a widespread change from single stem pruning to the multiple stem system at that time.

2.5.2.2.5. Source and concentration of conidia:

Nutman and Roberts (1960 a) preferred freshly extruded conidia from lesions on ripe berries for inoculation experiments because appressoria were produced soon after germination whereas conidia from green berries tended to have longer germ tubes often without appressoria. Bock (1956) found that a concentration above 10^5 conidia/ml ensured optimum berry infection in inoculation experiments, but Griffiths and Furtado (1972) used very small quantities of conidia (down to one conidium/berry) in a berry infection technique for assessing CBD. At very high concentration, germination may decrease (Firman & Waller, 1977). When conidia were germinated at concentrations of 186.3, 45.9 and 1.7×10^6/ml, Nutman and Roberts (1970) found that germination increased as the concentration decreased. In a washed series of dilutions there was more germination than in an unwashed set except at the highest conidial concentration. They suggested that maybe a natural germination inhibitor occurred in freshly extruded conidia which was readily removed on dilution with water.

2.5.2.2.6. Nutrients:

Compared with germination in water, various nutrients such as sugars in high dilutions increased the germination rate as did leachates and dilute extracts from coffee berries (Firman & Waller, 1977). They noted that this effect increased as the intrinsic viability of the conidia decreased. Thus conidial germination percentage of 57.5 was increased with the addition of 1% dextrose by 20% whereas one of 5.5 was increased by 610%. Improved nutrition also broadened the temperature range over which optimum germination occurred and raised the maximum to 35° C (Nutman & Roberts, 1960 b).

2.5.2.2.7. High dilutions of fungicides:

Nutman and Roberts (1962) found that a wide range of fungicidal chemicals at very high dilutions stimulated germination, growth and infectivity. This stimulation was independent of nutrition, but its degree was dependent on viability the relation being logarithmic and identical with that between viability and nutrition. It appeared that the degree of response to a fungicidal or nutritional stimulus was dependent on conidial viability and not on the nature of the stimulus.

2.5.2.2.8. Plant surface:

Nutman and Roberts (1960 a) showed that flowers, young berries and fully ripe fruits were highly susceptible to infection and that at least part of the variation in susceptibility between flowers and berries and between berries of different ages was the result of variation in germination rate of conidia on their surfaces. Probably nutrients diffuse more easily into infection droplets on flowers and ripe berries than on green berries. Steiner (1972) and Lampard and Carter (1973) found fungitoxic substances in the surface wax from berries of CBD resistant coffee cultivars.

2.5.2.2.9. Source of CBD inoculum and dispersal:

Conidia of C. coffeanum are produced in acervuli on the developing bark of young twigs and on diseased berries. Both conidial production (exudation) and dispersal are dependent upon water (Firman & Wailer, 1977). The role of conidia produced from twigs or berries in disease epidemiology was the subject of much debate during the 1960s. Initially it was considered that inoculum from the developing bark was the most important source (Nutman & Roberts, 1961) but later it was shown that diseased berries were by far the most potent source (Gibbs, 1969). In the absence of diseased berries, inoculum from the bark can initiate an epidemic but the subsequent progress of the disease is more dependent on conidia from the berries which account for most of the inoculum during most of the season (Griffiths et al., 1971).

2.6. Control of pathogen:

2.6.1. Use of resistant varieties :

Among arabica coffees such cultivars as Geisha and Blue Mountain have long been known to be rather resistant to CBD in Kenya whereas other types notably Harar, SL selections and Bourbon were particularly susceptible (Rayner, 1952). Blue Mountain, as a resistant cultivar was recommended for commercial use, but according to Gillette (footnote to Thorold, 1945) this cultivar would not produce economic crops when cultivated under heavy shade which was then common in most areas prone to severe CBD. Blue Mountain could be subject to heavy flower loss by CBD (Anon., 1958) even though the berries are resistant, so its yield can be raised by disease control (Bock, 1963). Rume Sudan and some progenies of Hybrido de Timor have high resistance to CBD but their quality is below acceptable East African standards (Anon., 1965; Fernie & Vermeulen, 1966). The cultivar K7 showed appreciable field resistance, almost as much as Blue Mountain

(Gibbs, 1968) and because of its quality, is quite widely grown in the middle and low coffee altitudes where it produces reasonable yields often without being sprayed.

Techniques for screening varieties for resistance have included the detached berry test (Bock, 1956) as well as field observations on natural disease incidence. More recently methods for screening young coffee seedlings for resistance based on inoculating the hypocotyl, cotyledons, sections of young stems and discs from seedling leaves have been developed (Cook, 1973). Hypocotyl infection of 4 - 6 week old seedlings is being used to detect disease resistance in the Kenya Coffee Breeding Programme (van der Vossen et al., 1976) but laboratory susceptibility has been found in some varieties showing apparent field resistance (Anon., 1975).

2.6.2. Use of fungicides:

By 1952 it was known that some degree of control of CBD could be exercised by fungicide sprays (Rayner, 1952) but little real success had been achieved. It was thought that a continuous protection of the developing fruit would be required. Bock and Rayner (1956) controlled the disease by monthly sprays of phenyl mercury acetate and fortnightly sprays of copper fungicides during the rainy seasons. However, the promising results with the mercury spray did not lead to practical recommendations because it was found to induce severe zinc deficiency in the coffee bush (Bock et al., 1958). Similar effects following arsenic sprays have been seen in Zaire (Bock et al., 1958).

Bock (1963) compared 25 different fungicide formulations containing various active principles in field trials and found that copper fungicides were the most effective whereas captan and maneb gave inconsistent results. Subsequently, Vermeulen (1968) screened 43 fungicides using tests to determine reduction of mycelial growth on

agar and prevention of conidial germination. Fourteen of those showing the best results were then tested in field trials. Results were variable but captafol was as good as copper.

Nutman and Roberts (1970) have described a laboratory technique for testing fungicides against CBD on the basis of their effect on sporulating capacity. A somewhat similar method was used by Vine et al., (1973 a). However, good antisporulants such as "Tuzet" were not effective against CBD (Griffiths and Gibbs, 1969; Gibbs, 1971) lending weight to the view that antisporulant activity which was the basis of early season spray schedules is not of major importance especially since it failed to work well even when overlapping crop was removed so that infected berries could not provide the initial inoculum (Gibbs, 1971).

Vine et al. (1973 a) tested some 60 fungicides in the laboratory for their ability not only to depress sporulation of C. coffeanum on the maturing bark of coffee but also to inhibit spore germination. The most promising were selected for field trials and 9 of them could be recommended for control of CBD in Kenya. These were 50% copper formulations, captafol, chlorothalonil, benomyl, thiophanate - methyl, thiophate, thiabendazole, dithianon and fentin hydroxide. Fentin hydroxide was later found less effective and is no longer recommended (Vine et al., 1973 b).

The advent of systemic fungicides produced many new materials which were very effective against CBD, including carbendazim and cypendazol (Baker, 1973; Okioga and Mulinge, 1974). However, Cook (1975 a) detected tolerance by the CBD pathogen of both of these benzimidazole fungicides in vitro and where they had been used continuously and exclusively, CBD control failed (Okioga, 1976). Tolerance to benomyl, also a benzimidazole fungicide, has occurred, and none, so far has been detected against the non-systemic protectant fungicide captafol. A few isolates obtained, in August 1976, from infected berries from Benlate sprayed plots were also found to be tolerant to Benlate (Javed, 1978).

Benlate (benomyl) a benzimidazole compound (methyl-1-butylcarbamoyl benzimidazol-2-ylcarbamate) was first recommended in Kenya in 1972 for the control of coffee berry disease (CBD) caused by the fungus Colletotrichum coffeanum Noack. In 1973 other fungicides were introduced for CBD control in Kenya. The fungicides were chemically similar to benomyl in that they were formulations of its degradation product, methyl benzimidazol-2-ylcarbamate (carbendazim, formerly referred to as MBC) (Clemons and Sisler, 1969; Maxwell and Broddy 1971) or of compounds similar to benomyl itself. These included Bavistin and Derosal (formulations of carbendazim) and Folcidin (Cypendazol methyl 1-5-cyanopentylcarbamoyl benzimidazol-2-ylcarbamate). In November 1974, Folcidin was withdrawn by its manufacturer because their screening trials at Upper Kiambu, Kenya, had indicated tolerance of C. coffeanum to this fungicide. The tolerant strains of C. coffeanum have now become endemic in high altitude coffee growing areas of Kenya after repeated use of benzimidazole fungicides (Benlate, Bavistin, and Derosal).

Fungicide tolerance has been reported by many workers. One of the earliest reports of tolerance to fungicides in fruit orchards was by Taylor (1953) who observed tolerance of apple root organisms to copper and sulphur fungicides. Apparent resistance of Venturia inaequalis to Dodine was noted by Szkolnik and Gilpatrick (1969) in New York apple orchards where Dodine gave unsatisfactory control of apple scab although it was previously used successfully for up to 9 years. Some cases of acquired and induced resistance to benomyl have been reported (Bollen and Scholten, 1971). This resistance was also found in Fusarium roseum (Hoitink and Schmithenner, 1970) and some laboratory resistance has been obtained for Fusarium oxysporum f.sp. lycopersici (Thanassoulopoulos et al., 1971). Bollen and Scholten (1971) noted that cyclamen soft rot caused by Botrytis cinerea was not controlled by benomyl. Goldberg and Cole (1973) reported a complete failure with benomyl applied to control Sclerotinia homoecarpa on a commercial golf-course. Tolerance to benomyl has been reported in Cercospora apii Fres (Berger, 1973) and C. beticola

(Georgopoulos and Dovas, 1973). In 1974 Wicks (1974) reported that Venturia inaequalis (CKE) Wint. was resistant to benomyl in South Australia. Subsequently, resistance to benomyl was reported from New Zealand (Tate and Samuels, 1976) in 1975. Benlate resistance was first reported in the U.S. in 1976 in Michigan (Jones and Walker, 1976). The problem was observed most frequently in orchards where benomyl was used regularly for at least 3 years. Since 1970, benzimidazole fungicides have been used extensively in Israel for the control of scab (caused by Venturia pirina Aderh.) in pear orchards. In 1975, resistance to these fungicides was found in V. pirina from two pear growing regions in Israel (Shabi and Ben Yaphet, 1976).

Geeson (1976) reported that the mycelial growth of 16 sensitive isolates of Botrytis cinerea was strongly inhibited by less than 1 µg/ml MBC with ED_{50} between 0.06 and 0.19 µg/ml. He noted that good growth, on agar media was shown by 26 fully tolerant isolates in the presence of 10,000 µg/ml MBC, although their production of sclerotia was delayed and in some cases reduced by high concentrations of MBC. He further observed that germination of conidia of sensitive isolates of B. cinerea was insensitive to MBC concentration which completely inhibited mycelial growth, but germ tubes were abnormal and did not develop further. Littrell (1974) reported that conidial measurements and microscopic observations of Cercospora arachidicola showed no evidence of reduction in size or change in morphology of conidia when the fungus was grown on benomyl-amended media. This is not in agreement with observations by Griffee (1973) with Colletotrichum musae in which conidial size was reduced by culturing on benomyl-amended medium.

Considering spore germination, hyphal growth, sporulation and stability of resistant isolates of Venturia pirina (Shabi and Katan, 1979) the same characters observed by Javed (1980) in C. coffeanum could be of epidemiological importance. Shabi and Katan (1979) reported that

resistant isolates of V. pirina were subcultured on fresh, fungicide free media about once each month for over I year, without loss of resistance. Dekker and Gielink (1979) reported that acquired resistance to pimaricin in Cladosporium cucumerinum and Fusarium oxysporum f. sp. narcissi was associated with decreased pathogenicity to cucumber seddlings and narcissus bulbs. This was in contrast to observations by Javed (1980) with C. coffeanum and by Shabi and Katan (1979) with V. pirina in which tolerant isolates were found as pathogenic as the sensitive isolates.

Benomyl tolerant Cladosporium carpophilum was isolated from peaches affected with scab in 1975 after benomyl was applied 4-7 times per year for 4 years in research plots (Chandler et al., 1978). They confirmed the persistence of tolerant strain in 1976 and 1977 and spraying experiments during both years using captan, maneb or sulphur in combination with benomyl were effective in controlling sensitive and tolerant strains of C. carpophilum and also the brown rot fungus, Monilinia fructicola. They further reported, in 1977, captafol alone or glyodin in combination with benomyl or captan were also effective against these diseases. Benomyl tolerant strains of Botrytis cinerea pers. ex pers. were obtained from all commercial glasshouse tomato crops samples in Lancashire in 1977, although benzimidazole fungicides had not been used on some nurseries for upto 3-4 years (Miller & Jeves, 1979). This was not in agreement with observations by Sutton (1978) with Venturia inaequalis in which combinations of benomyl with reduced rates of non-benzimidazole fungicides failed to control benomyl resistant isolates and the spread of resistant strains in North Carolina. Evidence that resistant isolates to a fungicide develop cross-tolerance has been demonstrated. Clark et al., (1974) reported that benomyl tolerant isolates of Cercospora arachidicola and C. personatum had cross-tolerance to a fungicide, thiophanate methyl, chemically related to benomyl. Georgopoulos and Dovas (1973) noted a serious outbreak of strains of Cercospora beticola resistant to benzimidazole fungicides in Northern Greece. Triphenyltin compounds were used exclusively until 1976 and 1977 growing seasons when the control of Cercospora leaf spot with

triphenyltin compounds was unusually inconsistent. Giannopolitis (1978) demonstrated cross-resistance to triphenyltin compound in benzimidazole resistant strains of C. beticola. Sozzi and Gessler (1980) found no difference between artificially induced MBC resistant mutants and MBC sensitive wildstrains of Fusarium lycopersici Snyd. et Hans. and Botrytis cinerea Pers. ex. Fr. when tested for their pathogenicity and fitness. Strains heterocaryotic for benomyl tolerance have been demonstrated in Botrytis cinerea Pers. (Pourtois et al., 1976).

The mechanisms by which living cells in general and fungi in particular acquire tolerance to a chemical include:-

(i) decreased membrane permeability

(ii) development of alternate metabolic pathways

(iii) inactivation of the chemical

(iv) alteration of the reactive site.

Hammerschlag and Sisler (1972) reported that respiratory inhibition by benomyl in sporidia of Ustilago maydis was not only dependent on toxicant concentration but also on cell concentration. They further observed that respiration of Saccharomyces pastorianus and S. cerevisiae seemed to be about as sensitive to benomyl as that of U. maydis.

Detection of benomyl tolerance in C. coffeanum before wide-spread losses occur and establishment of a range in tolerance is an advantage in the formulation of the disease control programme.

3. MATERIALS AND METHODS:

3.1. Isolation of benomyl sensitive and tolerant strains:

Infected green berries were harvested from benomyl treated plots at Migoa (altitude - 1676 m), Kibubuti (altitude - 1890 m) estates and from unsprayed plots at the Coffee Research Station (altitude - 1608 m.) in Kiambu District. The berries were surface sterilized with 1% calcium hypochlorite for five minutes then washed in five changes of distilled sterile water and incubated for 48 hr in a moist chamber for the fungus to sporulate. The chamber consisted of a plastic box 17 cm long, 11 cm wide and 5 cm high. Conidial suspensions were prepared from such cultures of Colletotrichum coffeanum and conidial density was estimated using a haemocytometer. For each isolate the concentration was later adjusted to 2×10^5 conidial/ml. The concentration of 2×10^5 conidial/ml for each isolate was diluted to give approximately 20 conidia/plate by using the plating method of Gibbs (1969) and Staples (1973). Benomyl at 10 µg/ml concentration was added to molten 3.4% malt extract agar (MEA) after cooling to 45°C before pouring into the inoculated plates. Approximately 20 ml of MEA was poured into each plate. Control plates contained MEA without benomyl. The plates were incubated for 10 days.

3.2. Pathogenicity of benomyl sensitive and tolerant strains:

The following conditions were observed when conducting pathogenicity tests. These included using 2×10^6 conidia/ml for inoculations; incubating inoculated detached berries at 20°C ± 0.5; recording percent infected berries at an interval of four days after inoculation for up to four weeks. All the treatments were replicated three times and each experiment was repeated once.

3.2.1. The effect of temperature on pathogenicity of benomyl sensitive and tolerant strains using detached berries of SL 28 coffee cultivar:

The boxes containing inoculated berries were incubated at 10, 15, 20, 25 and 30°C. Percent berries which developed lesions at the point of inoculation were recorded.

3.2.2. The effect of various conidial concentrations of benomyl sensitive and tolerant strains on detached berries of SL 28 coffee cultivar:

The following suspensions 10^3, 2×10^3, 5×10^3, 10^4, 2×10^4, 5×10^4, 10^5, 2×10^5, 5×10^5, and 2×10^6 conidia/ml were prepared. Two drops of each conidial suspension were put on detached berries in moist chambers and incubated. The procedure was repeated for all the other strains of C. coffeanum. Percent infected berries was recorded.

3.2.3. Mechanism of infection:

A detached berry inoculation technique (Bock, 1956) was used in this study. Expanding young, soft green berries were surface sterilized, placed on damp cellulose wadding in moist chambers and inoculated with one drop of conidial suspension for each strain. The inoculated berries were incubated at room temperature.

3.2.3.1. Epidermal scrappings of berries:

Four hours after inoculation, three epidermal scrappings were removed and put into McCartney bottles containing farmer's fluid (appendix I). This procedure was repeated for each strain after 8, 24 and 48 hr of inoculation. After 24 hr the epidermal scrappings were transferred to McCartney bottles containing lactophenol (appendix I).

Twenty four hours later the materials were removed from lactophenol and put into McCartney bottles containing acid fuchsin in lactophenol as given in appendix 1. After 24 hr of staining the materials were mounted in 50% glycerine on microscope glass slides, covered with cover slips and sealed using colourless nail polish. Observations were made under light microscope. Micro-photographs were taken.

3.2.3.2. Transverse sections of inoculated berries:

3.2.3.2.1. The portions of the berry pericarp, below the "inoculation drops", after 4, 8, 24 and 48 hr. of inoculation were fixed in Carnoy's fluid (appendix 2) for 24 hr. Sections of 12 μ thickness were cut using a microtome and stained with Heidenhain's iron-alum haematoxylin (appendix 2) and counter stained with Orange G in clove oil. Observations on fungal penetration were made under the light microscope.

3.2.3.2.2. Four hours after inoculation, sections of 12μ thickness were cut using a microtome and bleached in a mixture of boiling glacial acetic acid and 95% ethanol in a ratio of 1:1 V/V. The sections were then dipped in a solution of chloral hydrate and stained with 1% lactophenol cotton blue. The sections were examined under the light microscope for fungal penetration. This procedure was repeated at 8, 24 and 48 hr after inoculation for each strain.

3.2.4. Pathogenicity of benomyl sensitive and tolerant strains on detached berries of SL 28 coffee variety in the presence of benomyl:

Detached berries were inoculated using the technique developed by Bock (1956). Twenty five berries per replication of each treatment were surface sterilized and placed on dampened cellulose wadding in moist chambers. In one set of treatments all berries were punctured with sterile inoculating needle before placing different

benomyl concentrations. All 25 berries per replication were inoculated with one drop, of approximately 0.03 ml, of a given strain per treatment. The procedure was repeated for the various benomyl concentrations and for all the other strains. The moist chambers were incubated and the berries were examined for infection. Percent berries with active sporulation at the point of inoculation was calculated.

3.2.5. Determination of pathogenicity of benomyl sensitive and tolerant strains using hypocotyl infection tests:

Padang, Rume Sudan, Pretoria, K7, Catimor and SL 28 varieties were used for pathogenicity tests by inoculation of seedling hypocotyls as described by Cook (1973, 1976) and Cook and van der Vossen (1974). This technique is analogous to a preselection test applied in breeding programmes for resistance to Colletotrichum lindemuthianum in beans (Hubbeling , 1957; Mastenbroek, 1960). In this experiment 50 seeds of each variety were sown out, with parchment removed, in moist sterilized sand in plastic boxes with closely fitting transparent lids and kept at room temperature (20 - 24°C). The seedlings were inoculated within 5-6 weeks after sowing the seeds when the hypocotyls were approximately 3-5 cm long. A double inoculation at 48 hr interval was applied (Cook and van der Vossen, 1974). A temperature of about 22 - 24°C and relative humidity in the boxes of 100% were maintained for the first four days of incubation. This was followed by an incubation period at constant temperature of 19 - 20°C with lids removed from the boxes. At the end of three weeks from first inoculation the seedlings were individually scored for disease symptoms developed on the hypocotyl using a scale of 1 - 12 grades. The infection mean grades were calculated.

3.2.6. Determination of pathogenicity of benomyl sensitive and tolerant strains on berries on trees:

A field "attached-berry" inoculation test used by Bock (1956) and van der Vossen et al., (1976) was applied in this experiment. The following seven coffee varieties: Padang, Pretoria, Rume Sudan, K 7, Hybrido De Timor, SL 28 and SL 34 were used in the study. Artificial inoculation of berries on the trees was done at their most susceptible stage i.e. 12 - 14 weeks after flowering when the green berries were still soft (Mulinge, 1970 a). A total of 5-7 nodes with berries still at soft green stage of development on four branches per variety were enclosed in white plastic bags and after one day preconditioning sprayed with conidial suspension of a given strain. The same procedure was repeated for all strains. A double inoculation was applied at 48 hr interval to ensure maximum infection and the bags were removed four days after the first inoculation. A control branch was included per variety, where berries were bagged but sprayed with distilled sterile water instead of inoculum, to allow the assessment of natural infection. Percent infected berries was recorded at weekly intervals until six weeks after inoculation. This inoculation test was carried out in June-July 1981. The treatments were replicated three times.

3.3. Growth characteristics of the different strains in different media:

The main objective was to find a medium which would serve to distinguish benomyl sensitive from benomyl tolerant strains. Twenty seven media were tested. The basal composition of the culture medium contained plain agar (Oxoid), potassium, phosphate and magnesium sulphate except media 1, 2 and 15 which were plain agar (Oxoid), malt extract agar (MEA) and cherry agar respectively. The composition of basal medium was varied according to source of carbon, nitrogen and with or without additons of zinc sulphate, ferrous sulphate and copper sulphate as given in appendix 3. All media were adjusted to 5.2-6.8 range of pH.

The molten medium was cooled to 45°C before pouring into sterile petridishes of uniform size, 9 cm in diameter. The plates containing approximately 20 ml of medium were centrally inoculated with 5 mm diameter discs cut from the periphery of 10 day old MEA cultures. Each treatment consisted of three replicate plates for strains S, T_1 and T_2 for each medium and the experiment was repeated once. Two diameters of radial mycelial growth were measured in each of three replicates after 14 days of incubation and means calculated. Observations were made on mycelia pigmentation. Sporulation of benomyl tolerant (T_1 and T_2) and sensitive (S) strains was determined by cutting discs 18mm in diameter, from 21 day old sporulating cultures, macerating them using inoculating needle, agitating them in 10 ml distilled sterile water with a Griffin flask shaker for one minute. Determination of spore concentrations was done with aid of a haemocytometer and was expressed per mm^2.

3.4. Conidial germination and germ tube characteristics of benomyl sensitive and tolerant strains:

The following conditions, unless stated otherwise, were observed and included incubation of moist chambers at room temperature (21°C ± 4); harvested conidia from 10 day old cultures; examination of germination in a sample of not less than 300 conidia after 8, 24, 48 and 72 hr. All the treatments were replicated three times and the experiments repeated once.

3.4.1. The effect of conidial concentrations:

Suspensions ranging from 10^4 to 2×10^5 conidia/ml of the three selected strains, S, T_1 and T_2, were prepared. For each strain, 0.1 ml of conidial suspension was placed on a sterile cavity glass slide contained in a moist chamber. The moist chambers were incubated and examined for germination after 8, 24, 30, 48 and 72 hr. The rate of germination

during the lag phase of fungal growth and percent conidial germination were recorded.

3.4.2. The effect of washing conidia on germination:

A suspension of 10^5 conidia/ml was used. The conidia were divided into washed and unwashed lots. The conidia were washed using 400 ml of distilled sterile water and filtered through sartorius membrane filter with 0.8 μ pore size. A conidial suspension of 0.1 ml for each strain was put on a sterile glass cavity slide enclosed in a moist chamber and incubated. Periodic counts of germinated conidia were done after 8, 24, 30, 48 and 72 hr. Lag period of germination, germination rate and percent germination were noted and computed accordingly.

3.4.3. The effect of external source of glucose on percent germination:

Suspensions of 2×10^5 conidia/ml were prepared. Weighed amounts of glucose were dissolved separately in 100 ml distilled sterile water to give the following glucose concentrations: 1%, 2%, 3%, 4%, 5% and 6%. Three drops, of approximately 0.03 ml each, of conidial suspension for each strain and 3 drops of a given glucose concentration were put on a sterile glass cavity slide enclosed in a moist chamber and incubated. Control treatments for each strain had 3 drops of distilled sterile water added to 3 drops of conidial suspension/slide. In all treatments the final concentration for each strain was 10^5 conidia/ml while the final glucose concentrations were 0, 0.5, 1, 1.5, 2, 2.5 and 3%. Germination of conidia was examined after 72 hr of incubation. Total percent germination in each replication was calculated.

3.4.4. The effect of different benomyl concentrations on germination:

Suspensions containing 4×10^5 conidia/ml were prepared. Benomyl stock solutions at 2000, 200, 20 and 4 a.i. µg/ml were prepared. The slide germination method of evaluating fungicides (Anon., 1943 & 1947) was used and the following benomyl concentrations: 0, 0.25, 0.5, 1, 2, 2.5, 5, 10, 25, 50, 100, 125, 250, 500 and 1000 µg/ml were obtained. Periodic counts of germinated conidia were made. Lag period of germination, rate of germination and percent germination were computed.

3.4.5. The effect of temperature on germination of conidia and appressorial formation:

The conidia of three strains, S, T_1 and T_2, were subcultured on MEA only once from the active lesions of berries inoculated and incubated at $20^\circ C \pm 0.5$. Aqueous suspensions of 2×10^5 conidia/ml for each strain were prepared from the sporulating MEA cultures. For each strain, 0.1 ml conidial suspension was placed on a sterile cavity slide contained in a moist chamber. The moist chambers were incubated at 5, 10, 15, 20, 25 and $30^\circ C$ and periodic counts of germinated conidia were made.

3.4.6. The effect of different benomyl concentrations on appressorial formation:

The slide germination method of evaluating fungicides was used and the following benomyl concentrations: 0, 0.25, 0.5, 1, 2, 2.5, 5, 10, 25, 50, 100, 125, 250, 500 and 1000 µg/ml were obtained. Moist chambers were incubated at $20^\circ C \pm 0.5$. Germination of conidia was determined after 72 hr and observations made on germ tube characteristics.

3.4.7. Conidia viability:

Each strain was maintained on MEA plates amended with 0, 0.25, 0.5, 1, 2, 2.5, 5, 10, 25, 50, 100, 125, 250, 500 and 1000 µg/ml benomyl for 30 days. The conidia were harvested and aqueous suspensions containing 2×10^5 conidia/ml were prepared. For each strain, 0.1 ml conidial suspension was placed on a sterile glass cavity slide contained in a moist chamber and incubated. Periodic counts of germinated conidia were made after 72 hr.

3.5. Mycelial growth and sporulating capacity of benomyl sensitive and tolerant strains:

In all mycelial growth and sporulation experiments the following conditions were observed: 5 mm inoculum discs were cut from the periphery of 10 day old cultures; benomyl concentrations used were 0, 0.25, 0.5, 1, 2, 2.5, 5, 10, 25, 50, 100, 125, 250 , 500 and 1000 µg/ml; inoculated plates were incubated at room temperature, $21^{\circ}C \pm 4$; the treatments were replicated three times and the experiment repeated once.

3.5.1. The effect of different benomyl concentrations on mycelial growth:

Benomyl was added to MEA cooled to $45^{\circ}C$ before pouring into plates. Hyphal growth on benomyl amended MEA was determined by inoculating plates centrally with a given strain. The inoculated plates were incubated and colony diameters were measured. Two diameters of radial mycelial growth were measured after 14 days, in each of three replicates and the means calculated.

3.5.2. Sporulating capacity:

Two methods were employed.

3.5.2.1. Sporulation of the three strains was determined by inoculating plates with 0.1 ml of an aqueous suspension diluted to 200 conidia/ml. Benomyl was added to molten MEA after cooling to 45°C before pouring into the inoculated plates. Test plates were incubated for 21 days after which they were flooded with 10 ml of distilled sterile water and the conidial concentration ascertained with aid of a haemocytometer.

3.5.2.2. Sporulation of benomyl tolerant and sensitive strains was determined by cutting one 18 mm diameter discs from 21 day old sporulating cultures, macerating them using an inoculating needle and agitating them in 10 ml distilled sterile water using Griffin flask shaker for one minute. Conidial concentrations were determined with aid of a haemocytometer. Conidial size was also determined for each treatment in all replications.

3.6. The effect of subculturing benomyl tolerant strains:

3.6.1. Mycelial growth and sporulation:

Benomyl tolerant strains were transferred every two weeks on MEA without benomyl. After six months in culture 5 mm diameter agar discs, bearing young mycelium of the indicated strain, were cut from sporulating cultures and transferred to MEA containing different concentrations of benomyl. The benomyl sensitive strain was included as control. "Isolates" S, T_1 and T_2 which were subcultured only once on MEA were also included and labelled as SB, T_1B and T_2B. The inoculated plates were incubated for 14 days, colony diameters were measured in each of the three replicates and the means calculated. Sporulation was determined by cutting one 18 mm diameter discs from

21 day old cultures, macerating them using an inoculating needle and agitating them in 10 ml distilled sterile water using Griffin flask shaker for one minute. Conidial concentrations were determined with a haemocytometer.

3.6.2. Conidial germination:

Suspensions of 4×10^5 conidia/ml were prepared. "Isolates" S, T_1 and T_2 which were subcultured only once on MEA were included and labelled as SB, T_1B and T_2B. Various benomyl concentrations were prepared. Using the slide germination method conidia were incubated at $20^0C \pm 0.5$ in moist chambers. The final concentration in moist chambers was 2×10^5 conidia/ml. Conidial germination and germ tube characteristics were examined after 72 hr.

3.6.3. Berry infection:

Conidia of benomyl tolerant strains which were repeatedly subcultured for a period of six months, on fungicide free MEA, were used for berry inoculation. Benomyl sensitive strain was included as control. "Isolates" S, T_1 and T_2 which were subcultured only once on MEA were included and labelled as SB, T_1B and T_2B. After surface sterilization, 25 expanding soft green berries of SL 28 per replication of each treatment were placed on damp cellulose wadding in moist chambers. The berries were inoculated with 2 drops, approximately 0.03 ml each, of suspension of 2×10^6 conidia/ml. The moist chambers were incubated at $20^oC \pm 0.5$. Percent infected berries was recorded at an interval of four days from the day of inoculation for up to four weeks.

3.7. Anastomosis:

3.7.1. Conidial germination:

Agar slide cultures were used in this study. Suspensions of 200 conidia/ml of all the strains were prepared. Given amounts of 0.1 ml of each conidial suspension were placed on thin plain agar on microscope slides and spread over to allow distribution of the conidia. The slides were incubated and examined for germination and anastomosis of germ tubes after 4, 8, 24, 48 and 72 hr.

3.7.2. Hyphal growth:

MEA slides cultures and semi-permanent stained mounts, as described by Riddell (1950), were prepared for the three strains. Small thin squares were cut from a film of MEA and transferred to a sterile slide, inoculated on all four edges using a given strain, covered by a sterile cover slip, larger than the inoculated MEA blocks and incubated for 14 days. Each slide was placed on sterile curved glass rod enclosed in a sterile petri dish containing 20% aqueous glycerine. After incubation was completed, the cover slip was lifted vertically from the MEA preparation which was discarded. Two preparations of fungus which adhered to the glass slide and cover slip were mounted with a drop of 0.1% lactophenol cotton blue stain placed on to the centre of the fungal preparations. A slide was lowered carefully on to the cover slip preparation and a clean cover slip on to the slide preparation. These semi-permanent mounts were sealed at the edges with a colourless nail vanish and examined microscopically.

3.8 Cross tolerance:

3.8.1 Mycelial growth:

To test cross tolerance of benomyl tolerant strains, strains T_1 and T_2 were grown on MEA amended with Benlate (50% W.P), Bavistin (50% W.P) and Derosal (20% C.D.) at final concentrations of 0, 1, 10, 100, 250 and 1000 a.i. µg/ml. Benomyl sensitive strain,

S, was included as control. The fungicides were added to molten MEA after cooling to 45°C. The plates containing approximately 20 ml MEA were inoculated by placing 5 mm diameter discs of agar with young mycelium. Two diameters of growth of mycelium were measured after 14 days in each replication and the means calculated.

3.8.2. Sporulating capacity:

Sporulation of benomyl tolerant and sensitive strains was determined by cutting 18 mm diameter discs from 21 day old cultures and agitating them using Griffin flask shaker in 10 ml distilled sterile water for one minute. Conidial concentrations were determined with a haemocytometer.

3.9. Competitive behaviour of benomyl sensitive and tolerant strains:

Twenty five berries per replication of each treatment were surface sterilized and placed on damp cellulose wadding in moist chambers. Suspensions of 2×10^6 conidia/ml for each strain were prepared and mixed in a 1:1 ratio of S and T_1 ; S and T_2. The conidia of the strains used were initially harvested from active lesions of artificially inoculated berries and subcultured on MEA only once. The berries were inoculated with 2 drops of the conidial suspension of each mixture and incubated at 20°C ± 0.5. On the 14 th day after inoculation, when sporulation was abundant, conidia were harvested from each berry per replication. Suspensions of 200 conidia/ml were prepared. Plates were inoculated with 0.1 ml of the conidial suspension. Approximately 20 ml of molten MEA, cooled to 45°C, was poured in a given plate. The plates were incubated at room temperature for 10 days. To determine the number of growing colonies which were tolerant to benomyl, MEA plates containing 10 µg/ml benomyl were used. Benomyl amended MEA plates were centrally

inoculated with inverted 5 mm diameter agar discs bearing mycelium of the indicated colony of a given mixture cut from the periphery of sporulating colonies. The inoculated plates were incubated at room temperature and observations on hyphal growth were made after 14 days.

3.10. In vitro control of benomyl tolerant strains:

3.10.1. Effect of fungicides and their combinations on conidial germination of benomyl tolerant and sensitive strains:

Suspensions of 4×10^5 conidia/ml of strains S, T_1 and T_2 were prepared. The slide germination method was used and the following concentrations of Benlate (50% w.p.), Copper Nordox (50% w.p.) and captafol (80% w.p.): 0, 0.25, 1, 10, 100, 500 and 1000 a.i. µg/ml were obtained. Using different droppers 3 drops of each fungicide and 3 drops of each strain were put on sterile glass cavity slides enclosed in a moist chamber. As for fungicide mixtures 2 drops of benomyl and 2 drops of Copper Nordox or captafol at a given concentration were put on sterile glass cavity slides and 4 drops of a given strain added. In control treatments, where no fungicide was added, 3 drops of distilled sterile water were added to 3 drops of each strain. Thus in all treatments the final concentration was 2×10^5 conidia/ml. The moist chambers were incubated at room temperature. Counts of germinated conidia were made after 72 hr and percent germination calculated.

3.10.2. Effect of fungicides and their combinations on mycelial growth and sporulation of benomyl tolerant and sensitive strains:

The strains were grown on MEA amended with Benlate 50% w.p., Copper Nordox 50% w.p., captafol 80% w.p., half of Benlate and Copper Nordox and half of Benlate and captafol. All fungicides and their combinations were added after the autoclaved MEA was cooled to 45°C. The inoculated plates were incubated at room temperature

and colony diameters were measured after 14 days. Sporulation of benomyl tolerant and sensitive strains was determined by cutting 18 mm diameter discs from 21 day old sporulating cultures, macerating and agitating them in 10 ml distilled sterile water using a Griffin flask shaker for one minute. Conidial concentrations were determined with a haemocytometer.

4. RESULTS:

4.1. Isolation of the benomyl tolerant and sensitive strains of Colletotrichum coffeanum:

Evidence that the coffee berry disease (CBD) pathogen may have developed tolerance to benomyl was obtained from conidial germination followed by colony formation tests. Tolerant and sensitive strains of C. coffeanum were clearly differentiated when they were grown on MEA containing 10 µg/ml benomyl. Sensitive strains did not grow on this medium whereas tolerant strain grew readily. No tolerant strains were obtained from unsprayed plots at Coffee Research Station (CRS) Ruiru, and thus provided the sensitive S strain. Tolerant strains from Migaa and Kibubuti estates were designated T_1 and T_2 respectively (Table 1).

Table 1: Colony formation by C. coffeanum, strain S, T_1 and T_2, isolated from CRS, Migaa and Kibubuti estates on MEA amended with benomyl:

Fungicide	Rate µg/ml	Strain % colony formation		
		S	T_1	T_2
Benomyl	10	0	80	69

4.2. Pathogenicity of sensitive and tolerant strains:

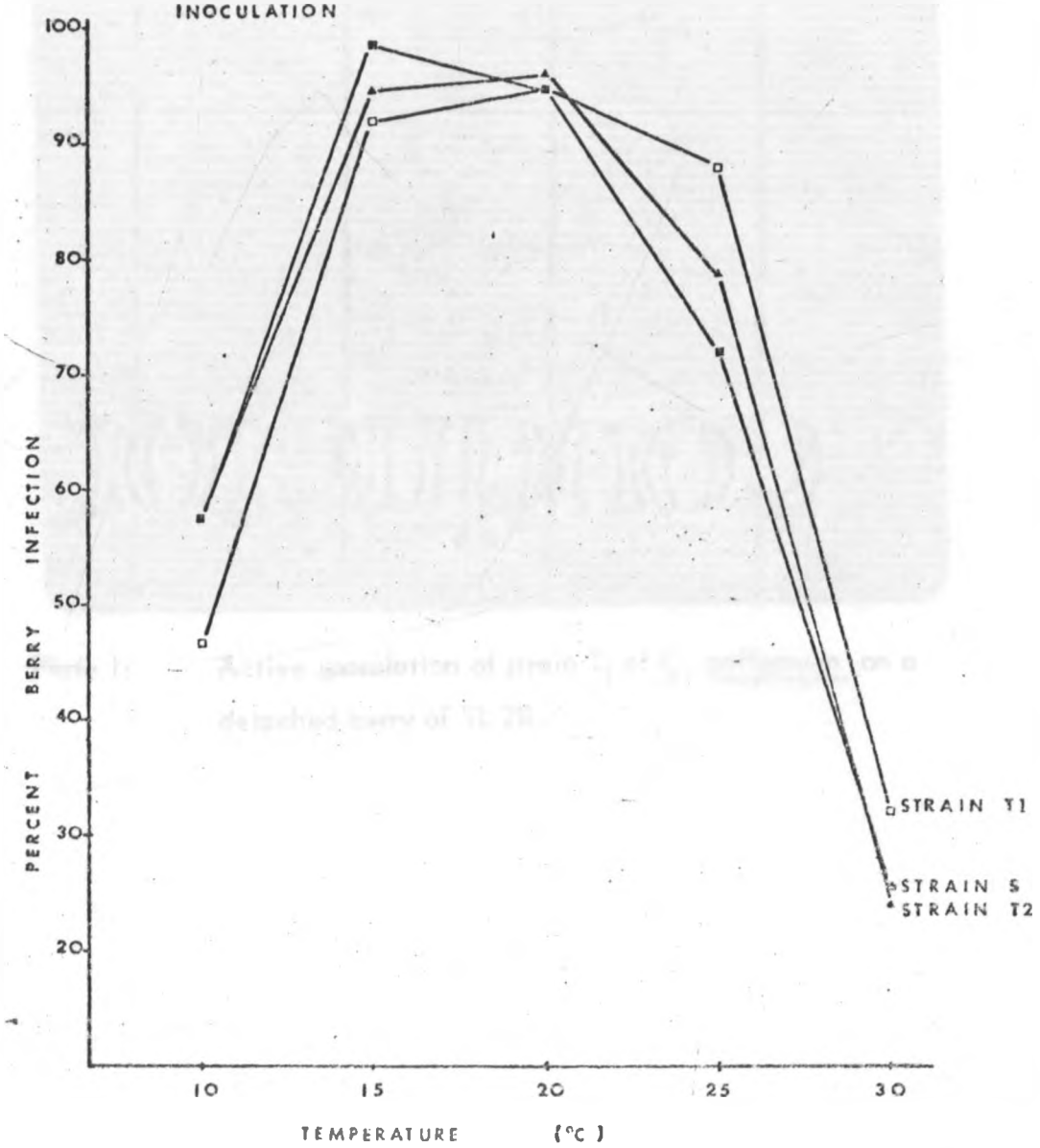
4.2.1. The effect of temperature on pathogenicity using berries of SL 28:

Fig 1 shows percent berry infection by the three strains at different temperatures after 25 days of incubation. Active conidial production was characterised by a pink mass of conidia on an initiated lesion on a detached berry as shown in Plate 1. At 10°C there was a delay in berry infection and a decrease in the rate of infection but once lesion formation occurred conidial production was not inhibited. At 15, 20 and 25°C, strains S and T_1 initiated berry infection at seven days after inoculation and there was active conidial production. Strain T_2 initiated berry infection at 20 and 25°C at seven days after inoculation and there was active sporulation. High percent berry infection and active sporulation for all strains was obtained at 15, 20 and 25°C. At 30°C, percent berry infection was highly reduced for all strains. At this temperature, the initiated lesion had no or greatly reduced active sporulation. CBD stalk infection was negligible. The analysis of variance table is given in appendix 4. There were significant differences at $P = 0.001$ among the temperatures, interaction between strains and temperatures, interaction between temperatures and time of recording and also among the different times of recording for berry infection. There were no significant differences at $P = 0.05$ among strains and also interaction between strains and time of recording for berry infection.

4.2.2. The effect of various conidial concentrations of benomyl sensitive and tolerant strains on detached berries of SL 28:

The effect of conidial concentration on berry infection is given in Fig 2. Plate 1 shows characteristic active sporulation with pink masses of conidia. Some infection occurred at a relatively low concentration of 10^3 conidia/ml but lesion formation was considerably

Fig. 1 : THE EFFECT OF TEMPERATURE ON PATHOGENICITY OF SENSITIVE (S) AND TOLERANT (T1, T2) STRAINS TO DETACHED BERRIES OF SL 28 COFFEE VARIETY AT THE 25th DAY FROM INOCULATION



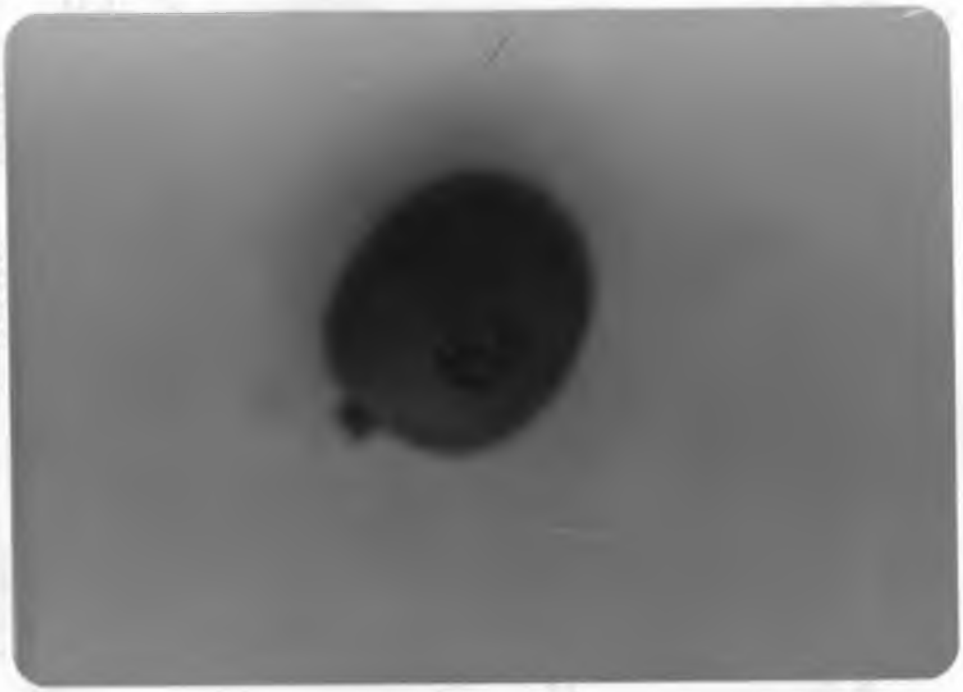
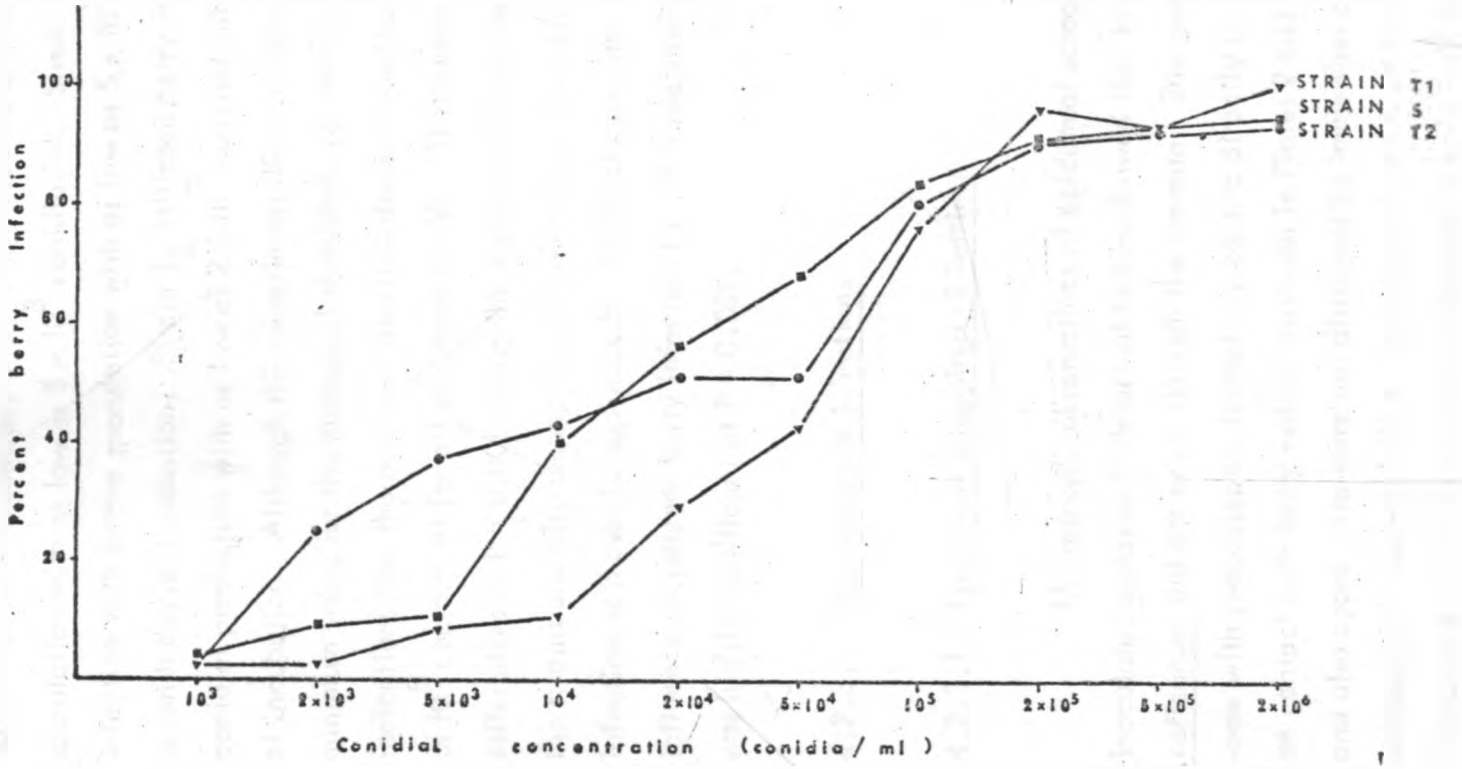


Plate I: Active sporulation of strain T_1 of C. coffeanum on a detached berry of SL 28.

Fig: 2: THE EFFECT OF CONIDIAL CONCENTRATION OF SENSITIVE (S) AND TOLERANT (T1, T2) STRAINS ON DETACHED BERRY INFECTION OF SL 28 COFFEE VARIETY AT THE 25th DAY FROM INOCULATION



less than at the higher conidial concentrations for all the strains. On the seventh day of incubation, strain S initiated berry infection with active sporulation with as low as 5×10^3 conidia/ml. Strain T_1 initiated berry infection with active sporulation with as low as 5×10^5 conidia/ml on the seventh day of incubation. Strain T_2 initiated berry infection with active conidial production with as low as 2×10^5 conidial/ml on the seventh day of incubation. Although this experiment did not indicate clearly a minimum value for the numerical threshhold of infection there were indications that the optimum infection density was above a concentration of 10^5 conidia/ml for all the three strains. There were significant differences at $P = 0.001$ among the various conidial concentrations, among the strains and also among the different times of recording for berry infection as shown in appendix 5. Conidial concentrations had great influence on percent berry infection by the three strains and this interaction was highly significant at $P = 0.001$.

4.2.3. Mechanism of infection:

4.2.3.1. Epidermal scrappings of berries:

Microscopic observations of epidermal scrappings from inoculated surfaces of detached berries showed that invasion by C. coffeanum did not occur through the stomata. The germinating conidia, some with pseudosepta, formed appressoria directly or on short germ tubes on contact with berry surface as shown in Plates 2 and 3. The conidia and also appressoria were not dislodged by immersion of berry surface scrappings in Farmers' fluid, lactophenol and Acid Fuchsin in Lactophenol. The appressorial formation was observed at epidermal junctures (Plate 2) and on epidermal cuticle (Plate 4). There was no observation of appressoria forming on the stomata. Some conidia of strain S germinated and formed appressoria at 4 hr compared to some conidia of strains T_1 and T_2 which germinated and formed appressoria at 8 and 24 hr of incubation respectively.

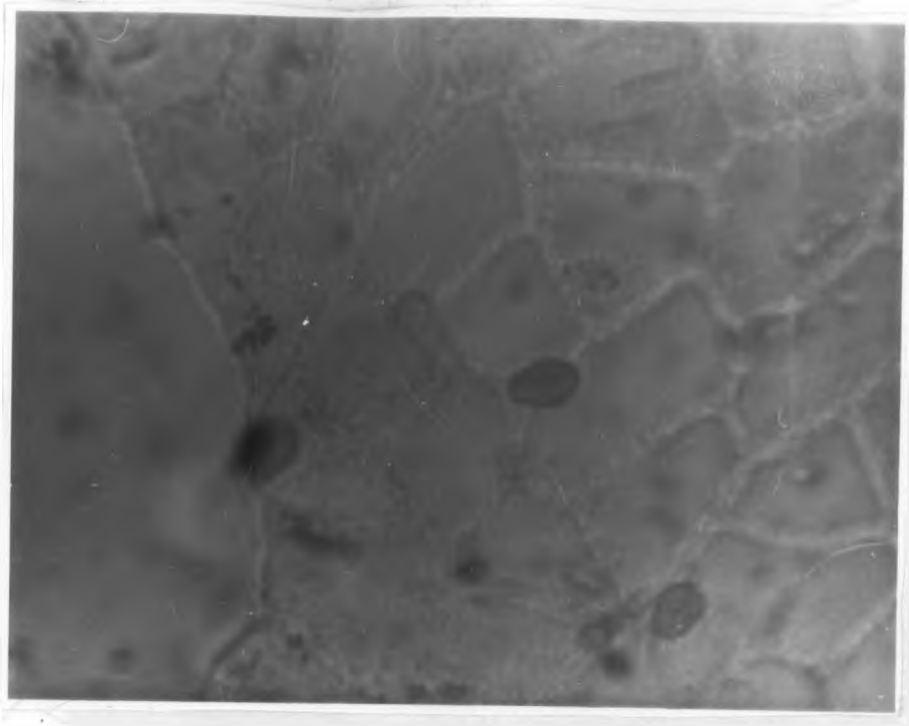


Plate 2: Conidial germination of strain S on epidermal junctures.

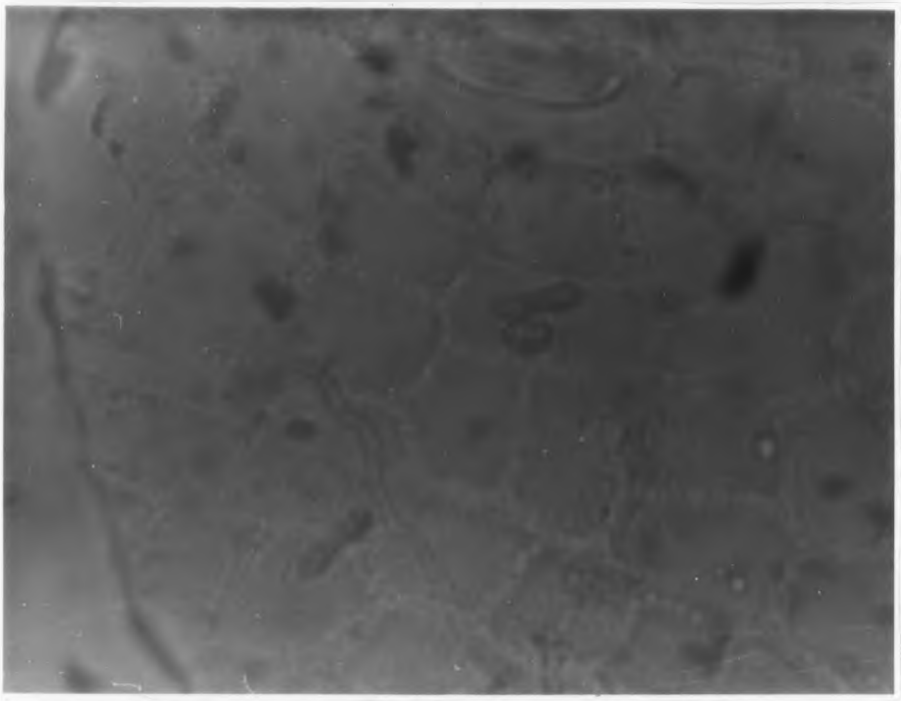


Plate 3: A germinating conidium of strain S of C. coffeanum with appressorium, without germ tube, on epidermal cell.

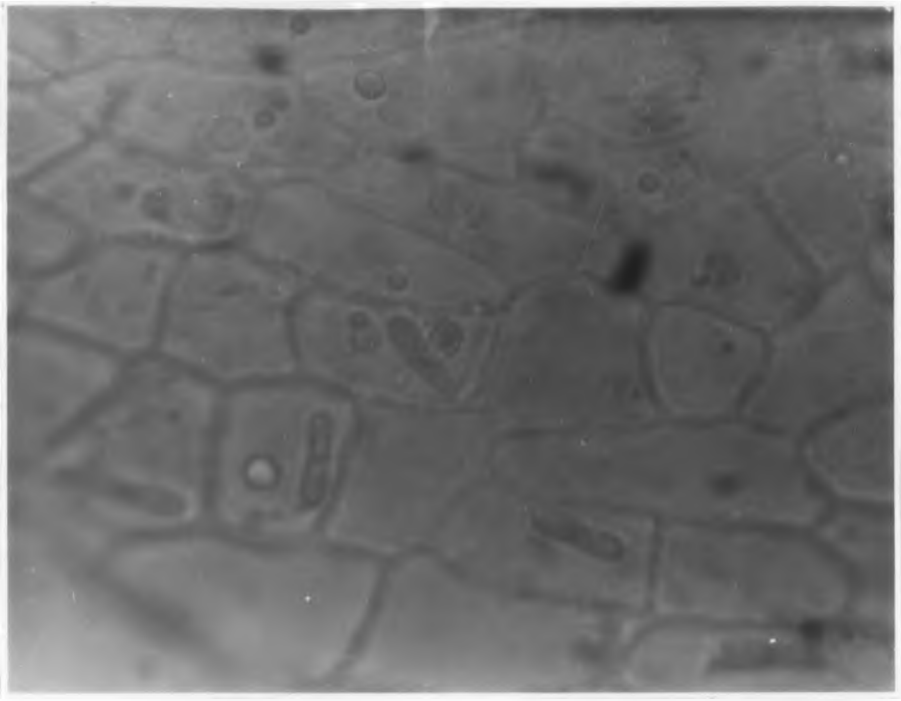


Plate 4: A germinating conidium of strain T_2 of C. coffeanum on coffee berry epidermal cell.

4.2.3.2. Transverse section of inoculated berries:

Penetration was presumably effected by infection hyphae which were produced at the lower surfaces of appressoria. The mode and time of penetration of infection hyphae into the berry pericarp was studied but the light microscope used did not reveal this vital information. The only observation made was attached conidia with appressoria on the surface of the pericarp.

4.2.4. Pathogenicity of benomyl sensitive and tolerant strains on detached berries of SL 28 in the presence of benomyl:

The effect of benomyl concentrations on pathogenicity of the three strains is given in Table 2 and Fig 3. Differences among strains in terms of berry infection under all fungicide concentrations, differences between fungicide concentrations over all strains and differences between time of recording infection over all strains were highly significant at $P = 0.01$. Interactions between strains and fungicide concentrations, between strains and time of recording infection and between fungicide concentrations and time of recording infection were also highly significant at $P = 0.01$ (Table 2 and appendix 6). All the three strains were highly pathogenic and had characteristic active sporulation with pink masses of conidia at the point of inoculation on detached punctured and unpunctured berries of SL 28 (Plate I). Generally, the punctured berries had higher berry infection compared with unpunctured berries for all strains (Table 2 b and appendix 6). Strain S gave lower levels of percent berry infection at higher benomyl concentrations compared with strains T_1 and T_2. Application of benomyl to the detached berries affected the rate of berry infection and reduced the number of berries showing active sporulation. This was particularly the case with strain S. At the seventh day after inoculation, the berries inoculated with strain S had active sporulation in 0.25 - 25 µg/ml benomyl treatments for punctured berries and unpunctured berries in up to 0.5 µg/ml benomyl. On the tenth day active sporulation

Table 2: Pathogenicity of benomyl sensitive and tolerant strains of C. coffeanum to detached berries of SL 28 coffee variety in the presence of benomyl:

(a) Table of means - % infection (CBD strains x days of recording)

CBD Strains	Days of recording (D)								Strain Means
	D_4	D_7	D_{10}	D_{13}	D_{16}	D_{19}	D_{22}	D_{25}	
S	0.00	2.80	16.22	30.98	44.27	53.16	59.11	64.40	33.87
T_1	0.00	12.98	46.44	57.78	67.78	75.07	81.60	83.56	53.15
T_2	0.00	4.80	35.24	51.42	59.64	68.67	74.98	79.69	46.81
Day means	0.00	6.86	32.64	46.73	57.23	65.63	71.90	75.88	

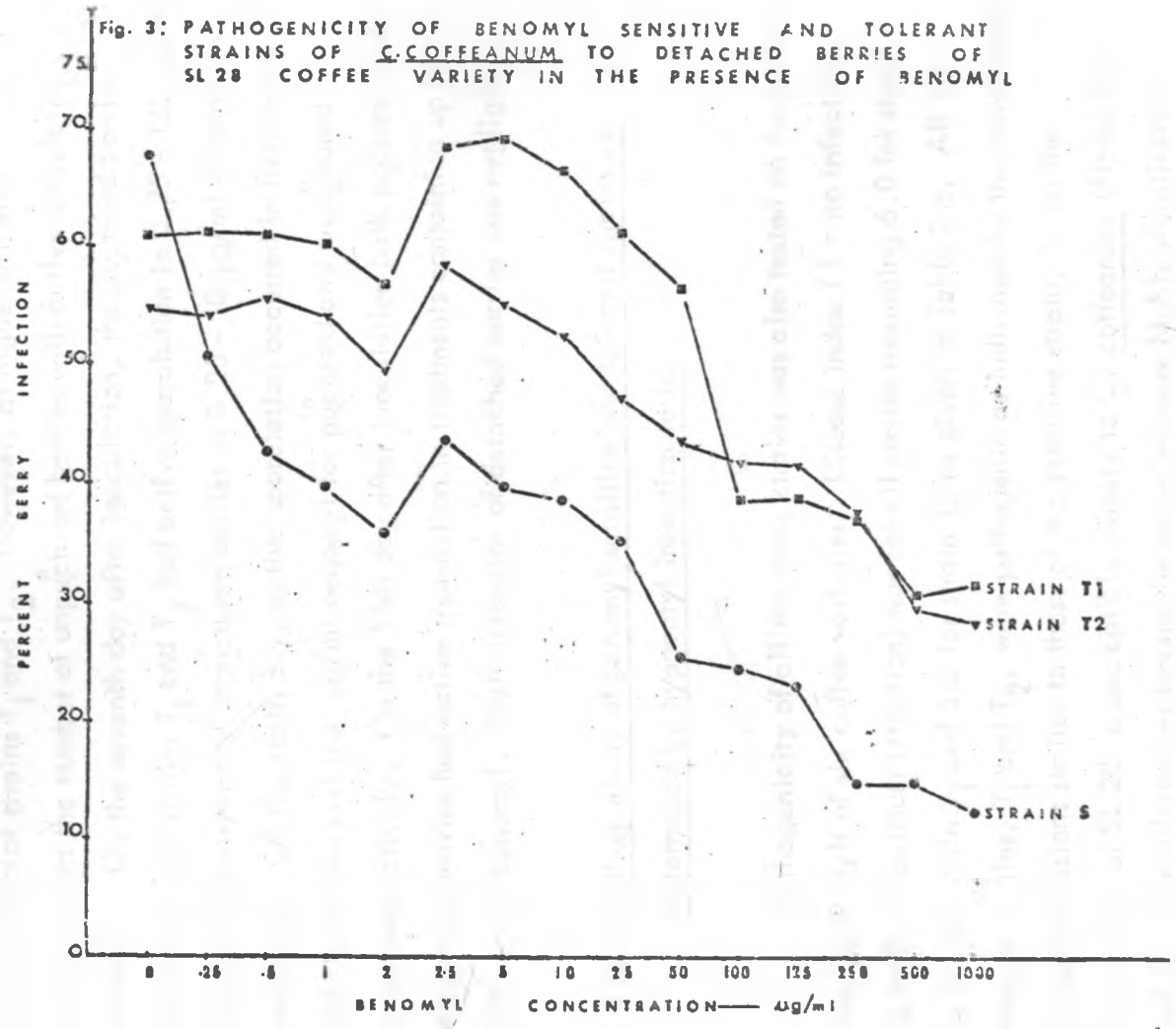
	5% level	1% level
L S D (Days)	2.97	3.91
L S D (Strains)	1.82	2.39

(b) Table of means - % infection (CBD strains x unpuctured/puctured berries)

CBD strains	Berries		Strain Means
	U	P	
S	18.10	49.63	33.87
T_1	40.14	66.16	53.15
T_2	28.26	65.36	46.81
U/P means	28.83	60.38	

	5% level	1% level
L S D (U/P)	1.49	1.96
L S D (Strains)	1.82	2.39

Fig. 3: PATHOGENICITY OF BENOMYL SENSITIVE AND TOLERANT STRAINS OF C.COFFEANUM TO DETACHED BERRIES OF SL 28 COFFEE VARIETY IN THE PRESENCE OF BENOMYL



occurred in treatments containing up to 100 µg/ml benomyl for punctured berries and in up to 25 µg/ml benomyl for unpunctured berries. On the 25th day after inoculation the punctured and unpunctured berries had active sporulation in treatments containing up to 1000 and 250 µg/ml benomyl respectively. Application of benomyl appeared to have little or no effect on the action of strains T_1 and T_2. However, at higher benomyl concentrations the number of unpunctured berries with active sporulation decreased. On the seventh day after inoculation, the punctured berries inoculated with strains T_1 and T_2 had active sporulation in 0.25 - 125 µg/ml benomyl treatments and unpunctured berries in 0.25 - 10 µg/ml benomyl treatments. On the tenth day, active sporulation occurred in treatments with up to 1000 and 125 µg/ml benomyl for punctured and unpunctured berries respectively. On the 25th day after inoculation both punctured and unpunctured berries had active sporulation in treatments containing up to 1000 µg/ml benomyl. Stalk infection of detached berries was negligible.

4.2.5. Pathogenicity of benomyl sensitive and tolerant strains as determined by hypocotyl infection tests:

Pathogenicity of all the three strains was also tested on fungicide free hypocotyls of six coffee varieties. Disease index (1 = no infection up to 12 = maximum infection) was for all strains averaging 6.0 for strain S, 6.1 for strain T_1 and 5.8 for strain T_2 as given in Table 3 a. All the tolerant strains, T_1 and T_2, were pathogenic as indicated by the development of typical lesions similar to those of the sensitive strain, S, on the hypocotyls of SL 28, a susceptible variety to C. coffeanum (Plates 5 a and b). The differences among the varieties were highly significant at P = 0.001. The interaction between strains and varieties and differences among strains in terms of symptom development over all varieties were not significantly different at P = 0.05.

Table 3: Pathogenicity of benomyl sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum on hypocotyls of different coffee varieties:

(a) Infection mean grades

VARIETIES	STRAINS S	T_1	T_2	Variety means
Pretoria	6.3	6.3	4.5	5.7
Padang	4.4	4.3	4.2	4.3
K 7	4.9	4.6	4.6	4.7
Catimor	4.3	5.0	4.7	4.7
Rume Sudan	4.7	4.3	4.8	4.6
SL 28	11.7	11.6	12.0	11.8
Strain Means	6.1	6.0	5.8	

	5% level	1% level
L S D (Varieties)	0.94	1.26
L S D (Strains)	0.66	0.89

(b) ANOVAR TABLE

	SS	df	MS	F value
Reps	2.13	2	1.07	1.1233 N.S.
Varieties	371.20	5	74.24	78.3235***
Strains	0.74	2	0.37	0.3917 N.S.
Var x strains	7.71	10	0.77	0.8129 N.S.
Error	32.23	34	0.95	
Total	414.01	53		

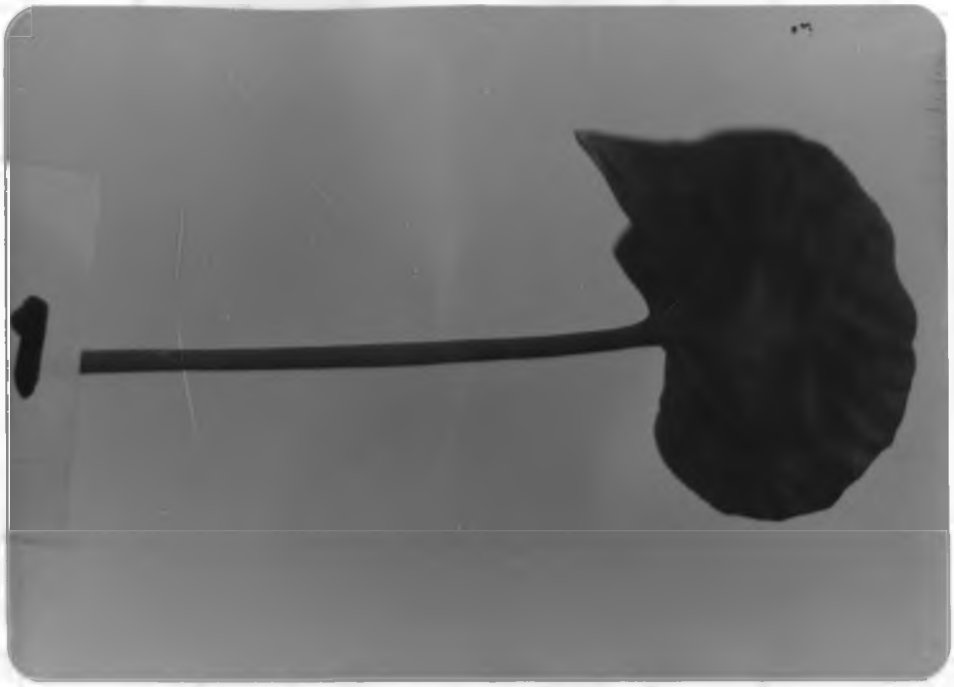


Plate 5 a: Grade 1 of the disease index used for recording CBD infection on hypocotyls of the coffee seedlings.

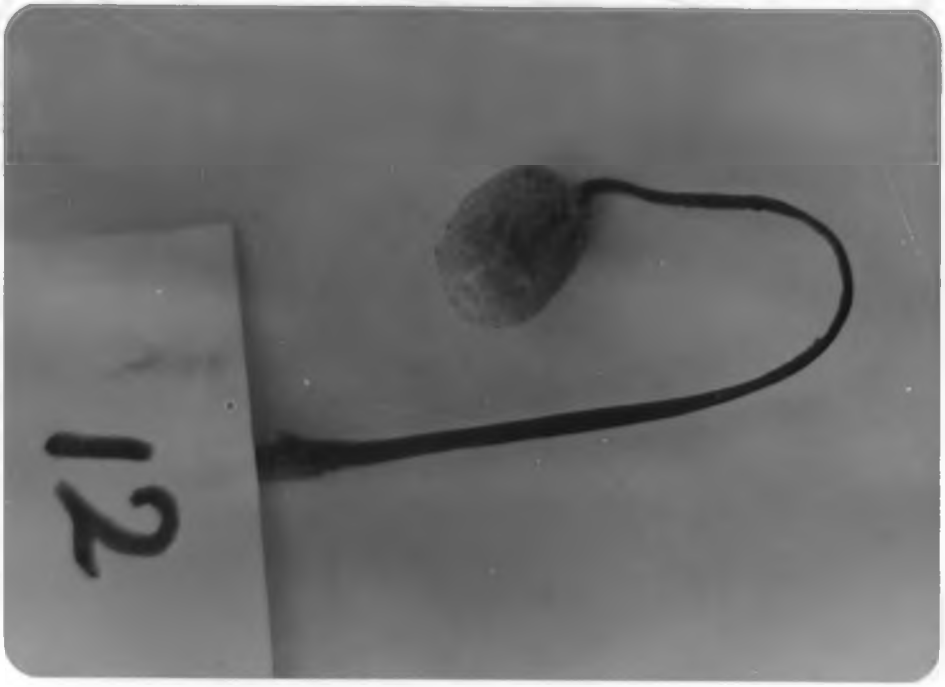


Plate 5 b: Grade 12 of the disease index used for recording CBD infection on hypocotyls of coffee seedlings.

Fig 4: VARIATIONS IN PATHOGENICITY OF BENOMYL SENSITIVE (S) & TOLERANT (T_1 & T_2) STRAINS OF C. COFFEANUM ON BERRIES OF DIFFERENT COFFEE VARIETIES

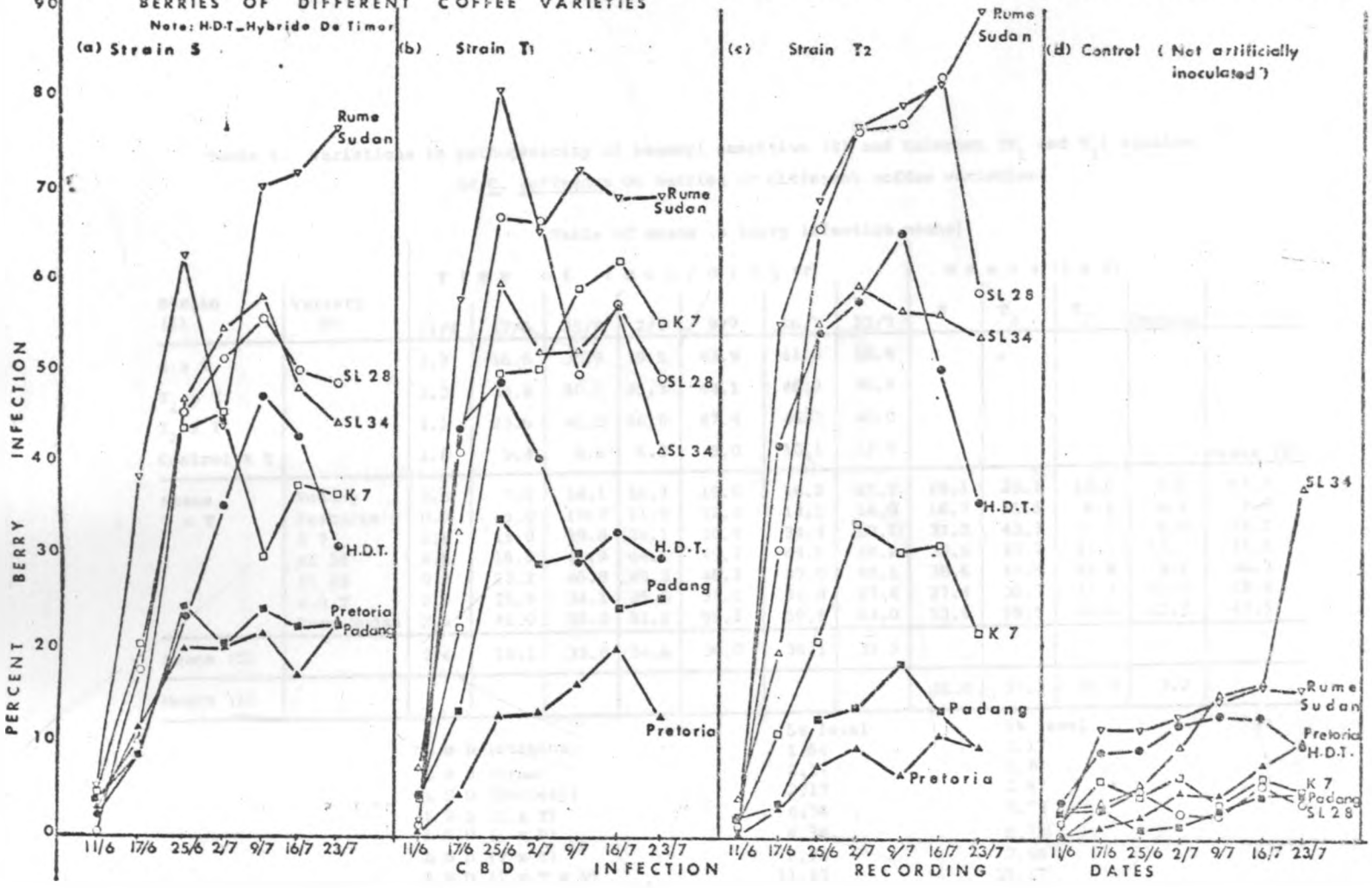


Table 4: Variations in pathogenicity of benomyl sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum on berries of different coffee varieties:

Table of means (% berry infection means)

Strain (1)	Variety (V)	Time of recording (T) 11/6	17/6	25/6	2/7	9/7	16/7	23/7	means (1 x V) S	T_1	T_2	Control	Means (V)
S x T		3.3	16.6	38.2	39.5	43.9	41.6	40.4					
T_1 x T		3.3	30.8	50.5	45.3	44.1	46.2	40.5					
T_2 x T		1.1	23.6	40.8	46.8	47.8	46.7	40.0					
Control x T		1.8	5.4	5.6	6.8	8.0	10.1	12.9					
Means V x T	Padang	3.3	7.1	18.1	16.3	19.0	16.2	15.7	28.1	23.1	10.6	2.9	13.7
	Pretoria	0.9	5.0	10.7	11.9	12.3	14.1	14.0	16.7	11.5	6.8	4.4	9.8
	K 7	2.8	15.0	29.8	34.1	30.8	34.4	29.7	31.2	43.3	21.5	4.8	25.2
	SL 34	4.5	16.5	41.9	44.1	45.7	44.6	44.2	38.0	43.2	43.7	13.1	34.5
	SL 28	0.4	23.2	45.8	49.3	46.2	49.0	40.1	38.6	47.5	55.9	3.2	36.3
	H.D.T	2.6	25.9	34.1	35.6	38.5	34.8	27.4	27.4	32.7	43.7	10.0	28.4
	Rume Sudan	2.1	41.0	56.0	51.1	59.2	59.8	63.0	53.6	59.5	64.6	12.3	47.5
Means (T)		2.4	19.1	33.8	34.6	36.0	36.1	33.5					
Means (1)									31.9	37.3	35.3	7.2	

	5% level	1% level
L S D (Strains)	1.54	2.17
L S D (Time)	2.17	2.87
L S D (Variety)	2.17	2.87
L S D (1 x T)	4.34	5.73
L S D (1 x V)	4.34	5.73
L S D (V x T)	5.74	7.58
L S D (1 x T x V)	11.47	15.17

4.2.6. Pathogenicity of benomyl sensitive and tolerant strains on mature trees:

A summary of the results of field scores for coffee berry infection is given in Fig 4 and Table 4. Summary of the prevailing weather conditions such as temperature, relative humidity and rainfall at the time when the experiment was in the field is given in appendix 7. Differences among strains in terms of berry infection among coffee varieties and also among the times of recording were highly significant at $P = 0.01$. All the three strains caused characteristic field coffee berry disease (CBD) symptoms on the inoculated berries (Plate 6). Rume Sudan variety gave exceptionally high scores of percent berry infection when inoculated with the three strains. Strain T_2 gave lower percent berry infection when used for inoculating Padang, Pretoria and K 7 varieties compared to strains T_1 and S. Strain T_2 gave higher percent berry infection when used for inoculating Hybrido De Timor variety compared to strains T_1 and S. There were some variations in overall mean percent berry infection among the strains when used for inoculating SL 28 and SL 34 varieties. The overall percent means for berry infection of SL 28 and SL 34 varieties were 36.46% and 35.73%, 39.8% and 41.92% and 40.16% and 45.88% for strains S, T_1 and T_2 respectively. In some cases percent berry infection of the varieties declined possibly due to berry fall after CBD infection.

To determine if tolerance was retained or lost upon infection of the host plant, conidia from lesions produced by the tolerant strains, T_1 and T_2, were tested on benomyl amended MEA. Mycelia produced by the new conidia were found tolerant at 10 µg/ml benomyl as those of the original inoculum, thus tolerance was transmitted throughout consecutive infections of berries.

Plate 6: Coffee berry disease symptoms on green berries under field conditions.

4.3. Growth characteristics of the different strains in different media:

4.3.1. Mycelial growth characteristics:

Data on radial mycelial growth are given in Tables 5 a, b and c and appendix 8. On the 27 different media used, the radial mycelial growth was not identical in the three strains. On most of the the media the three strains were similar in appearance. The change from white to any other colour appeared to coincide with the formation of the first conidia. Pigmentation and radial mycelial growth were recorded in two week old cultures. The mycelial growth of all strains was characterised by very sparse blackish mycelial mat in medium 1 where only agar (Oxoid) was used. Strains T_1 and S had very limited radial mycelial growth of 30 mm and 29.2 mm respectively compared with 39.5 mm for strain T_2. All strains had compact mycelial growth in medium 2 which was MEA. Black grey, with blue tint, coloration of mycelia with a whitish growth margin was observed in all strains. All strains grew readily in this medium giving 77.3 mm, 79 mm and 78.7 mm diameters of fungal colony of S, T_1 and T_2 respectively. All strains had grey black to black mycelia with indistinct growth margins in medium 15 which was the coffee berry extract. All strains had extensive non compact mycelial mats. Strain T_2 had radial mycelial growth measuring 80.3 mm compared to 73.8 mm and 78.7 mm for strains S and T_1 respectively and the difference was highly significant at $P = 0.01$ as shown in appendix 8 a.

4.3.1.1. Influence of carbon source on mycelial growth:

The mycelial growth of all strains were more compact in media containing simple carbohydrates compared to the more complex ones. The pigmentation produced by the three strains when different media were used showed slight variation from the coloration observed when the strains were grown on MEA. The most marked colour variations were observed when the three strains were grown on media 20 and 21. When medium 20 was used, a brown color was produced by the three strains below the grey to blue grey mycelial mat. Slight colour variations were observed when

the three strains were grown on medium 21. A pinkish colour was produced by the three strains in their central mycelial mat and the mycelial colour changed gradually to dark grey then blue to a white conspicuous growth margin. A brown colour was also produced by the three strains below the mycelial mat.

Table 5 a: Radial mycelial growth of strains S, T_1 and T_2 on four sources of carbon

Radial mycelial growth (mm)

Strain	Glucose	Sucrose	Starch	Cellulose	Total	Mean
S	45.86	57.75	55.58	48.47	207.66	51.92
T_1	62.72	62.61	53.70	40.89	219.92	54.98
T_2	59.53	63.19	50.56	45.61	218.89	54.72
Total	168.11	183.55	159.84	134.97	646.47	
Mean	56.04	61.18	53.28	44.99		53.87

Se (carbon) 3.26

Se (interactions) 5.65

Se (strains) 2.83

Radial mycelial growth of the three strains was most extensive on media containing sucrose. Radial mycelial growth of strains T_1 and T_2 was more extensive on media containing glucose compared to strain S

which had slightly limited radial mycelial growth. Reduced radial mycelial growth in strains T_1 and T_2 was observed on media containing starch and cellulose compared to the more extensive radial mycelial growth of strain S.

4.3.1.2. Influence of nitrogen source on mycelial growth:

All the strains were capable of utilizing organic and inorganic nitrogen. However, utilization of nitrogen was varied.

Table 5 b: Radial mycelial growth of strains S, T_1 and T_2 on three sources of nitrogen:

Strain	Radia mycelial growth (mm)				
	Potassium nitrate	Ammonium nitrate	Peptone	Total	Mean
S	49.21	37.98	68.56	155.75	51.92
T_1	56.42	40.48	68.04	164.94	54.98
T_2	53.60	40.44	70.13	164.17	54.72
Total	159.22	118.90	206.73	484.86	
Mean	53.08	39.63	68.91		53.87

Se (nitrogen) 2.83

Se (interactions) 4.90

Se (strains) 2.83

Radial mycelial growth of the three strains of the same fungus varied somewhat on different culture media. Reduced radial mycelial growth was observed on media containing inorganic nitrogen while media containing organic nitrogen had enhanced mycelial growth. Radial mycelial growth was much more affected when the strains were growing on media containing ammonium nitrate (media 7, 8, 9, 10, 20, 21, 22 and 23) as the source of nitrogen compared to potassium nitrate (media 3, 4, 5, 6, 16, 17, 18 and 19) and the difference was highly significant at $P = 0.01$ as shown in appendix 8 a.

4.3.1.2. Influence of heavy metals, viz. zinc, iron and copper, on mycelial growth of strains S, T_1 and T_2

Table 5 c: Radial mycelial growth of strains S, T_1 and T_2 on media with or without zinc, iron and copper:

Strain	Radial mycelial growth (mm)			
	Heavy metals		Total	Mean
	+	-		
S	48.83	55.00	103.83	51.92
T_1	52.08	57.88	109.96	54.98
T_2	49.56	59.89	109.45	54.73
Total	150.47	172.77	323.24	
Mean	50.16	57.99		53.87

Se (Heavy metals) 1.63

Se (interactions) 4.00

Se (strains) 2.83

Reduced mycelial growth for all strains was observed on media containing zinc sulphate, ferrous sulphate and copper sulphate while media without these elements had enhanced mycelial growth and the difference was highly significant at P = 0.01 as shown in appendix 8 a.

4.3.2. Sporulation:

Significant differences in conidial production were found among the strains and also among the formulated media at P = 0.001. Some media enhanced conidial production of the strains while the others depressed it and this interaction between strains and media was highly significant at P = 0.001 as shown in the appendix 10 b. Generally, all strains sectored in culture. The sectors differed from the original strains mainly in colour which ranged from pink to light pink then to white compared to the normal blackish grey colour as shown in Plate 7. Occasionally distinct zonation occurred on mycelial mat of these strains as shown in Plate 8.

4.3.2.1. Influence of carbon source on sporulation of the three strains:

Table 6 a: The influence of carbon source on sporulation of strains S, T_1 and T_2

Strain	Conidia / mm^2 x 1000					
	Glucose	Sucrose	Starch	Cellulose	Total	Mean
S	21.14	23.19	17.02	5.77	67.12	16.78
T_1	25.22	28.62	16.59	4.12	74.55	18.64
T_2	27.47	29.27	19.72	4.46	80.92	20.23
Total	73.83	81.08	53.33	14.35	222.59	
Mean	24.61	27.03	17.78	4.78		18.55

Se (carbon) 1.41

Se (interactions) 2.50

Se (strains) 1.22

Plate 7: Sectoring of strain T_1 of *C. coffeanum* on malt extract agar.

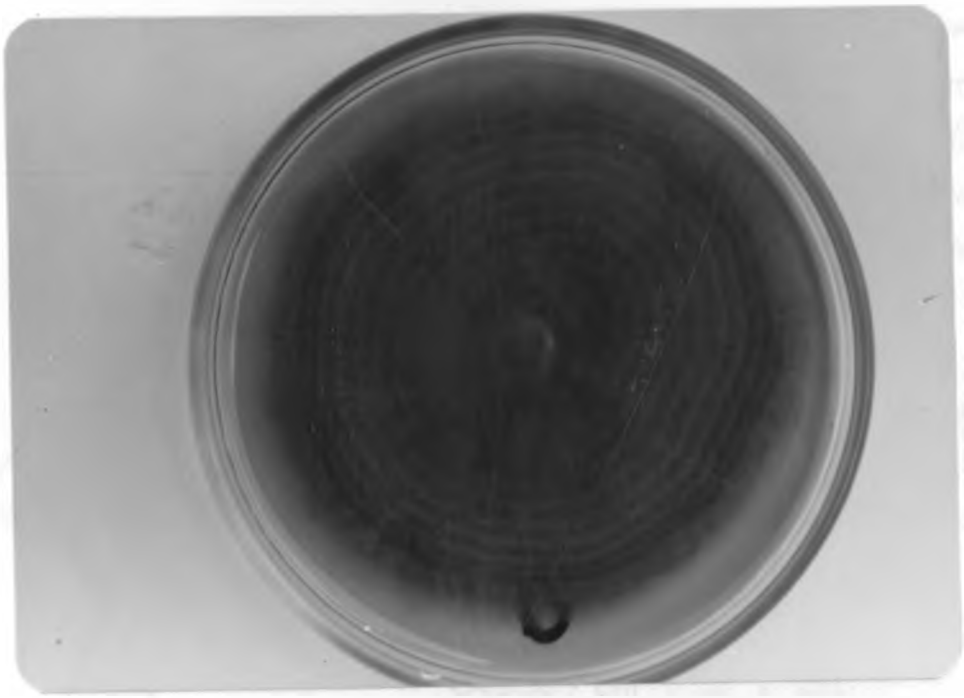


Plate 8: Zonation of strain T_2 of C. coffeanum on malt extract agar.

Conidial production in all the strains was enhanced in media containing simple carbohydrates, glucose and sucrose, compared to the more complex ones, starch and cellulose (Table 6 a). In all strains higher numbers of conidia/mm^2 were recorded in media 3, 4, 7, 8, 11, 12, 16, 17, 20, 21, 24 and 25 containing glucose and sucrose compared to media 5, 6, 9, 10, 13, 14, 18, 19, 22, 23, 26 and 27 containing starch and cellulose as shown in appendix 10 a. Various carbon sources affected conidial production of all the strains differently and the difference among the carbon sources was highly significant at $P = 0.001$ (appendix 11). Differences in conidial production among the strains was significant at $P = 0.05$. The interaction between carbon sources and conidial production of strains was not significant at $P = 0.05$.

4.3.2.2. Influence of nitrogen source on sporulation of the three strains:

Table 6 b: The influence of nitrogen source on sporulation of strains S, T_1 and T_2:

Strain	Conidia / mm^2 x 1000				
	Potassium nitrate	Ammonium nitrate	Peptone	Total	Mean
S	17.93	11.41	21.01	50.35	16.78
T_1	19.13	10.16	26.62	55.91	18.64
T_2	21.18	11.30	28.20	60.68	20.23
Total	58.24	32.87	75.83	166.94	
Mean	19.41	10.96	25.28		18.55

Se (nitrogen) 1.22

Se (interactions) 2.11

Se (strains) 1.22

In all strains the conidial production was reduced in media containing inorganic nitrogen while in media containing organic nitrogen sporulation was enhanced (Table 6 b). In all strains higher numbers of conidia/mm^2 were recorded in media 11, 12, 13, 14, 24, 25, 26 and 27 containing peptone compared to media 3, 4, 5, 6, 16, 17, 18 and 19 and media 7, 8, 9, 10, 20, 21, 22 and 23 containing potassium nitrate and ammonium nitrate respectively (Table 6 b and appendix 10 a). Sporulation was much more reduced when all the three strains were grown in media containing ammonium nitrate compared to media containing potassium nitrate. The differences among nitrogen sources were highly significant at P = 0.001. The interaction between strain and media was not significant at P = 0.05 (appendix 11).

4.3.2.3. Influence of heavy metals, viz iron, zinc and copper, on sporulation of three strains:

Table 6 c: Sporulation of strains S, T_1 and T_2 on media with or without zinc, iron and copper:

	Conidia / mm^2 / ml x 1000			
	Heavy metals		Total	Mean
Strain	+	-		
S	17.31	16.26	33.57	16.79
T_1	20.26	17.02	37.28	18.64
T_2	20.81	19.65	40.46	20.23
Total	58.38	52.93	111.31	
Mean	19.46	17.64		18.55

Se (Heavy metals) 0.70
Se (Interactions) 1.72
Se (strains) 1.22

Enhanced conidial production for all strains was observed on media containing zinc sulphate, ferrous sulphate and copper sulphate while media without these elements had reduced sporulation. The difference between media with or without these elements was significant at $P = 0.05$. The interaction between strain and media was not significant at $P = 0.05$ (appendix II).

4.4. Conidial germination and germ tube characteristics of sensitive and tolerant strains:

4.4.1. The effect of conidial concentrations on germination:

The effect of conidial concentrations on germination is given in Table 7. Conidia from the benomyl sensitive strain (S) and tolerant strain (T_1 and T_2) showed variation in germination. Strain S had 75 - 80% germination at 72 hr compared to 30 - 36% and 25 - 29% for strains T_1 and T_2 respectively. Percent germination in all strains increased as conidial concentration increased. For instance at 72 hr, strain S had 49%, 57.6%, 60.5%, 67.8%, 74.7%, strain T_1 showed 7.8%, 10.4%, 19.1%, 27%, 31.9% and strain T_2 showed 5.9%, 9%, 18.2%, 20.7%, 28.3% germination at 10^4, 2×10^4, 5×10^4, 10^5 and 2×10^5 conidia/ml respectively. Lag period of germination was also influenced by conidial concentration although generally the benomyl tolerant strains showed a delay in germination. At 24 hr, strain S showed germination at all conidial concentrations compared to strain T_1 and T_2 which showed no germination at 10^4 conidia/ml but showed low percent germination at all other conidial concentrations. At 8 hr, strain S showed germination at all conidial concentrations compared to strain T_1 for which no germination was recorded at 10^4, 2×10^4, 5×10^4 conidia/ml except at 10^5 and 2×10^5 conidia/ml. Strain T_2 showed no germination at 8 hr. Conidial concentration and time of incubation influenced percent germination of all strains and their interaction was highly significant at $P = 0.01$. The overall percent germination means for interactions between conidial concentration and

Table 7: The effect of conidial concentration on t germination of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Strain (1)	Hours of Incubation (T)	(C) Spore concentration /0.1 ml 1000	2000	5000	10,000	20,000	Means I x T	Means strain
S	8	2.4	4.12	7.64	18.15	22.05	10.87	
	24	15.67	18.35	33.55	39.17	40.95	29.54	
	30	21.65	24.55	36.05	42.14	42.99	33.48	
	48	41.06	44.72	50.08	57.46	66.18	51.90	
	72	48.94	57.58	60.49	67.81	74.73	61.91	
Means (I x C)		25.95	29.86	37.56	44.95	49.38		37.54
T_1	8	0.00	0.00	0.00	0.97	1.59	0.51	
	24	0.00	0.63	0.93	4.37	3.45	1.88	
	30	1.92	0.39	5.71	14.43	15.88	8.27	
	48	4.92	7.80	12.72	17.50	21.41	12.87	
	72	7.02	10.35	19.08	27.02	31.94	19.24	
Means (I x C)		2.93	4.44	7.69	12.86	14.85		8.55
T_2	8	0.00	0.00	0.00	0.00	0.00	0.00	
	24	0.00	0.39	0.78	1.91	4.83	1.58	
	30	1.89	2.53	5.16	6.64	9.52	5.15	
	48	4.02	5.51	8.71	12.51	16.25	9.40	
	72	5.93	6.98	18.19	20.68	28.25	16.41	
Means (I x C)		2.37	3.48	6.57	8.35	11.77		6.51
Means (C x T)	8	0.8	1.37	2.55	6.38	7.88		Means (T) 3.80
	24	5.22	6.46	11.75	15.15	16.41		11.00
	30	8.49	10.16	15.64	21.07	22.80		15.63
	48	16.67	19.35	23.83	29.15	34.61		24.72
	72	20.90	25.64	32.59	38.50	44.97		32.52
Means (Conc)		10.42	12.59	17.27	22.05	25.33		

	5% level	1% level
L S D (Strains)	0.36	0.47
L S D (Spore Conc. and Time)	0.46	0.61
L S D (Time x Conc.)	1.03	1.35
L S D (I x Conc. and I x Time)	0.80	1.05

strains were 25%, 29.9%, 37.6%, 45% and 49.4% for strain S; 2.9%, 4.4%, 7.7%, 12.9% and 14.9% for strain T_1; and 2.4%, 3.5%, 6.6%, 8.4% and 11.8% for strain T_2 at 10^4, 2×10^4, 5×10^4, 10^5 and 2×10^5 conidia/ml respectively. Strain S showed 10.9, 29.5, 33.5, 51.9 and 61.9 overall percent germination means compared to 0.5%, 1.9%, 8.3%, 12.9% and 19.2% for strain T_1 and 0%, 1.6%, 5.2%, 9.4% and 16.4% for strain T_2 at 8, 24, 30, 48 and 72 hr respectively.

4.4.2. The effect of conidia washing on germination:

The effect of conidia washing on germination is given in Table 8 and Fig. 5. Percent germination in all strains was affected by washing of conidia. Strain S showed 44.4 and 15.1 as overall percent germination means compared to 13.3% and 5.6% for strain T_1 and 8.5% and 3.1% for strain T_2 where conidia were unwashed and washed respectively. At 72 hr, strain S showed 69.4% and 41.3%, strain T_1 showed 26.6% and 14.4% and strain T_2 showed 20.1% and 7.4% conidial gemination for unwashed and washed conidia respectively(Fig. 5). These differences between unwashed and washed conidia were highly significant at $P = 0.01$. Conidia washing did not affect the lag period of germination of strains S and T_2 although, generally, strain T_2 showed a delay in germination. Strain S showed germination at 8 hr while strain T_2 showed germination at 24 hr for both unwashed and washed conidia. Strain T_1 showed germination at 8 hr and 24 hr for unwashed and washed conidia respectively.

4.4.3. The effect of external source of glucose on percent germination:

The effect of external source of glucose on germination is given in Fig 6. Washed conidia from benomyl tolerant (T_1 and T_2) and sensitive (S) strains showed variation in total percent germination at 72 hr. In distilled sterile water total percent germination of conidia for all strains was lower compared with germination in various dilutions of glucose as shown in Fig 6 and the differences were highly significant at $P = 0.01$.

Table 8: The influence of conidia washing on germination (%) of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Strain (1)	Time (Hrs)	(W) spore unwashed	Washing washed	Means (1 x T) S	T_1	T_2	
S x washing		44.41	15.14				
T_1 x washing		13.32	5.56				
T_2 x washing		8.47	3.10				
							Means (Hrs)
Washing x Hrs	8	6.76	0.38	9.94	0.78	0.00	13.57
	24	15.26	3.73	23.02	4.00	1.47	9.50
	30	20.95	5.14	25.22	9.68	4.22	13.04
	48	28.65	9.40	35.37	12.26	9.46	19.03
	72	38.70	21.01	55.31	20.46	13.78	29.85
Means Washing		22.07	7.93				
Means strains				29.77	9.44	5.79	

	5% level	1% level
L S D (Strains)	0.55	0.73
L S D (Washing)	0.47	0.59
L S D (Time)	0.71	0.94
L S D (Hrs x W)	1.0	1.33
L S D (1 x Hrs)	1.22	1.63
L S D (I x W)	0.77	1.03

Fig.5: THE INFLUENCE OF CONIDIA WASHING ON GERMINATION (%) OF SENSITIVE (S) AND TOLERANT (T1 , T2) STRAINS OF C. COFFEANUM

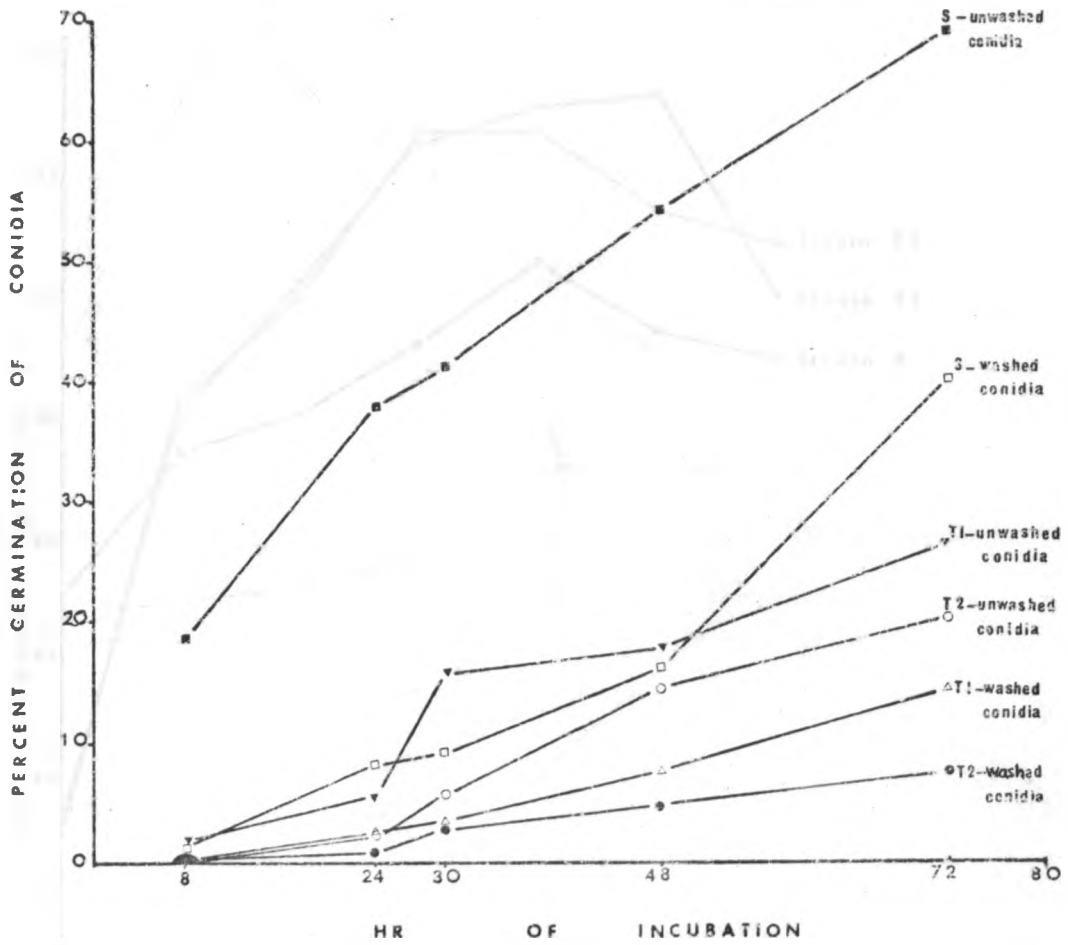
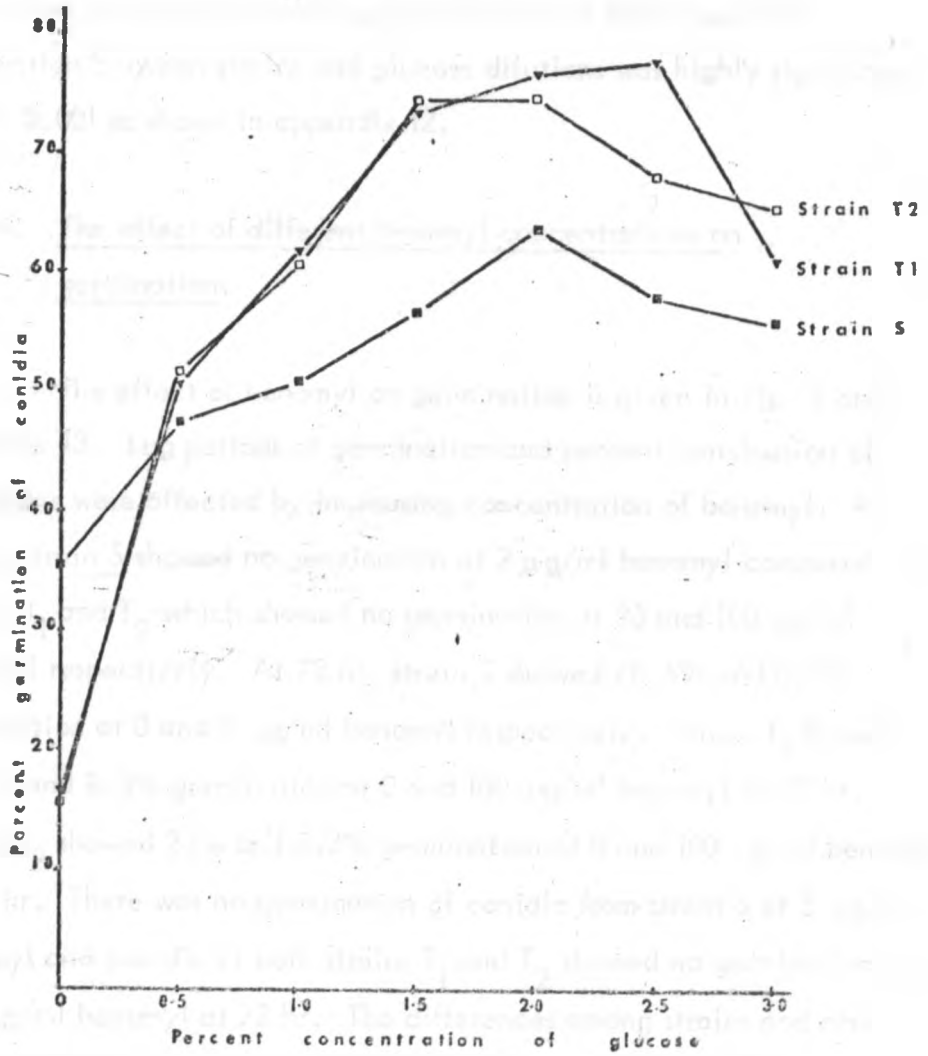


Fig. 6: THE EFFECT OF EXTERNAL SOURCE OF GLUCOSE ON PERCENT GERMINATION OF CONIDIA OF SENSITIVE (S) AND TOLERANT (T1, T2) STRAINS OF C. COFFEANUM



Total percent germination of strain S conidia increased with addition of glucose to 2% concentration and then declined. Strain T_1 showed increased total percent germination with increase of glucose concentration to 1.5% stabilized at 2% and 2.5% glucose concentration and declined at 3% glucose concentration. Strain T_2 showed increased total percent germination with increase in glucose concentration to 1.5%, stabilized at 2% and declined in total percent germination starting from 2.5% glucose concentration. There were significant differences at P = 0.001 among strains and also various glucose dilutions. Glucose dilutions had significant effect on conidial germination of all strains and this interaction between strains and glucose dilutions was highly significant at P = 0.001 as shown in appendix 12.

4.4.4. The effect of different benomyl concentrations on germination:

The effect of benomyl on germination is given in Fig. 7 and appendix 13. Lag periods of germination and percent germination of all strains were affected by increasing concentration of benomyl. At 24 hr, strain S showed no germination at 2 µg/ml benomyl compared to strains T_1 and T_2 which showed no germination at 25 and 100 µg/ml benomyl respectively. At 72 hr, strain S showed 78.5% and 0.9% germination at 0 and 2 µg/ml benomyl respectively. Strain T_1 showed 30.2% and 8.3% germination at 0 and 100 µg/ml benomyl at 72 hr. Strain T_2 showed 23% and 5.3% germination at 0 and 100 g/ml benomyl at 72 hr. There was no germination of conidia from strain S at 5 µg/ml benomyl and conidia of both strains T_1 and T_2 showed no germination at 500 µg/ml benomyl at 72 hr. The differences among strains and also benomyl concentrations were highly significant at P = 0.01. Benomyl concentrations had tremendous influence on conidial germination of all strains and this interaction was highly significant at P = 0.01 (appendix 13).

Plates 9a, b, c and d show the type of germination encountered

Fig. 7: THE EFFECT OF BENOMYL ON GERMINATION OF STRAINS S, T1 AND T2 OF C. COFFEANUM AT 72 HR.

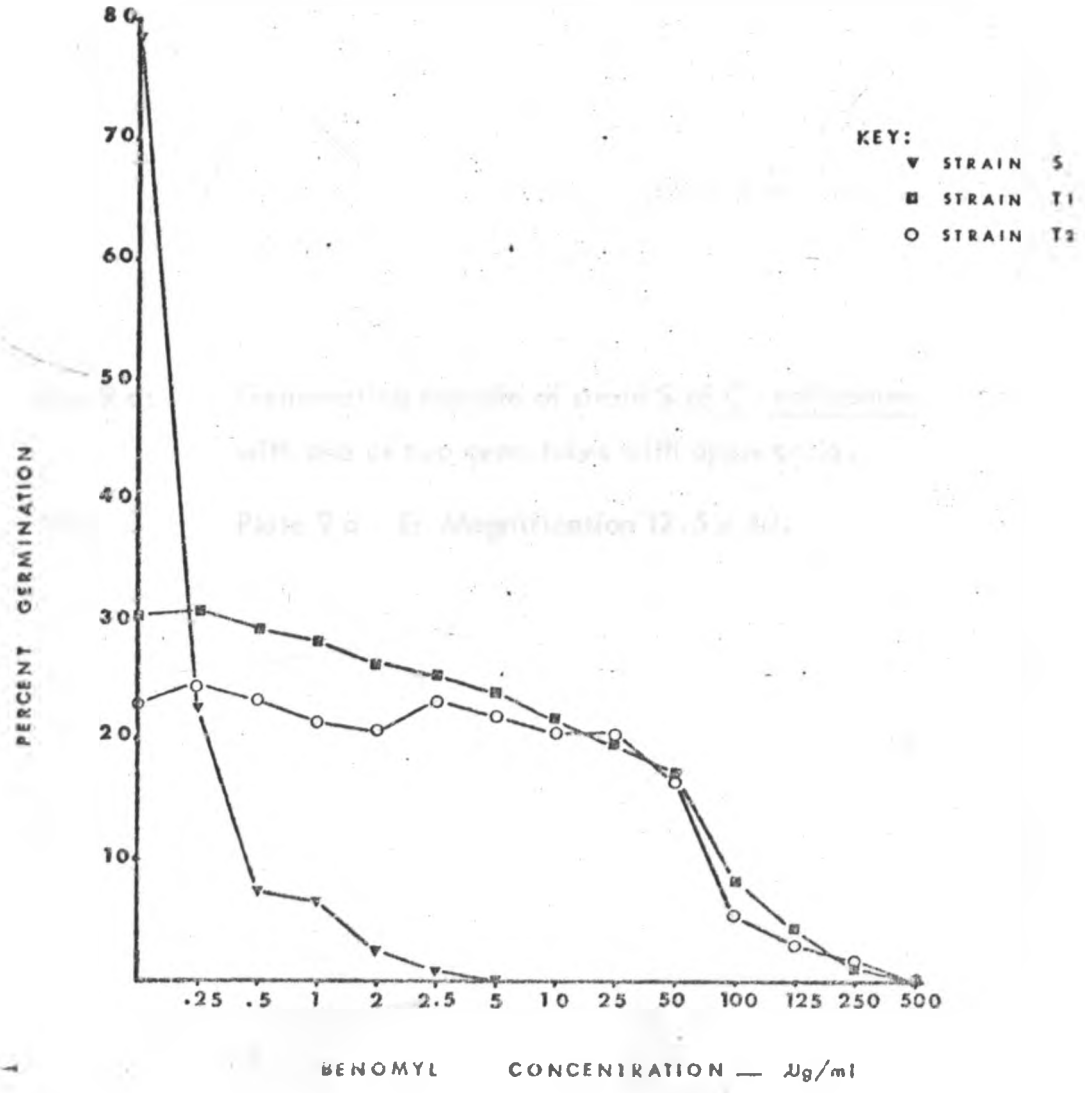


Plate 9 a: Germinating conidia of strain S of C. coffeanum with one or two germ tubes with appressoria.

Note: Plate 9 a - f: Magnification 12.5 x 40.

Plate 9 b: Germinating conidia of strain S of C. coffeanum with germ tubes bifurcated forming more than one appressorium.

Plate 9 c: Germinating conidium of strain T_2 of C. coffeanum with appressorium without germ tube.

Plate 9 d: Germinating conidium of strain T_1 of C. coffeanum with more than two germ tubes.

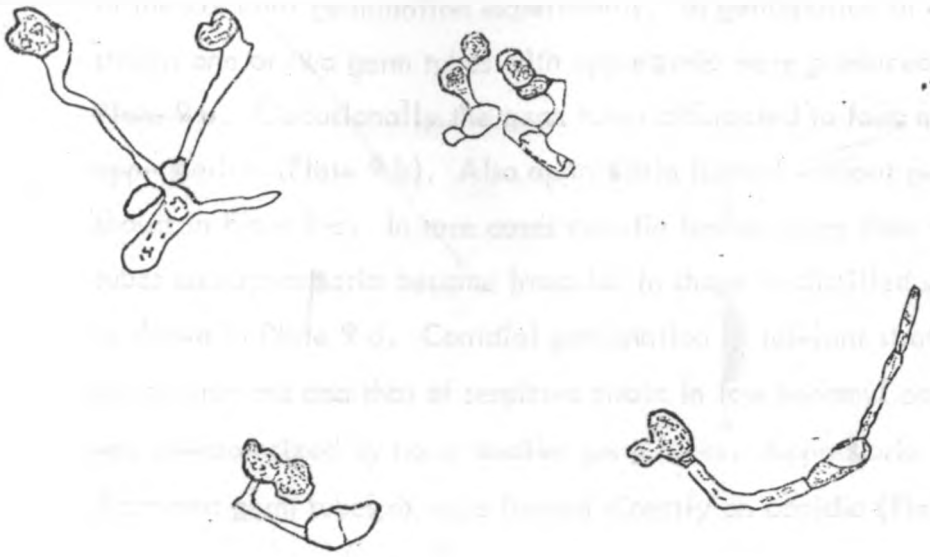


Plate 9 e: Germinating conidia of strain T_1 of C. coffeanum with bilobed appressoria

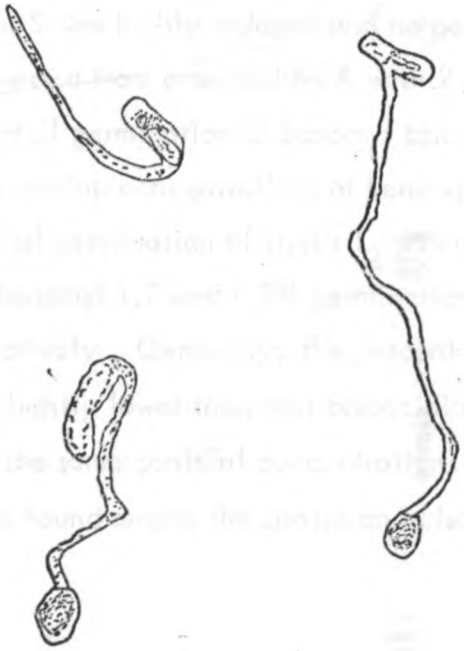


Plate 9 f: Germinating conidia of strain T_2 of C. coffeanum with long germ tubes with or without appressorium

in the conidial germination experiments. In germination of conidia of all strains one or two germ tubes with appressoria were produced as shown in Plate 9 a. Occasionally the germ tubes bifurcated to form more than one appressorium (Plate 9 b). Also appressoria formed without germ tubes as shown in Plate 9 c. In rare cases conidia formed more than two germ tubes and appressoria became irregular in shape in distilled sterile water as shown in Plate 9 d. Conidial germination of tolerant strains in high concentrations and that of sensitive strain in low benomyl concentrations was characterized by no or smaller germ tubes. Appressoria formed on the shortened germ tubes or were formed directly on conidia (Plate 9 c).

4.4.5. Conidia viability:

Summarised data on this experiment is given in Table 9. Germination of benomyl tolerant strains (T_1 and T_2) conidia harvested from 30 day old non-amended MEA cultures was more or less 50% less than that of benomyl sensitive strain (S) from similar non-amended MEA plates. In the presence of benomyl in MEA, germination of harvested conidia of strain S was highly reduced and no germination was observed for conidic harvested from amended MEA with 2.5 or more µg/ml benomyl. Conidial germination of benomyl tolerant strains was not inhibited by increasing concentrations of benomyl. However, at 500 µg/ml benomyl, conidial germination of strain T_2 was reduced considerably and at 1000 µg/ml benomyl 1.7 and 1.2% germination was recorded for strains T_1 and T_2 respectively. Generally, the percent conidial germination of all strains was slightly lower than that of conidia harvested from 10 day old cultures and at the same conidial concentration. Highly significant differences were found among the strains and also benomyl concentrations at $P = 0.01$.

4.4.6. The effect of temperature on germination of conidia and appressorial formation:

Varying temperatures affected lag period, germination rate,

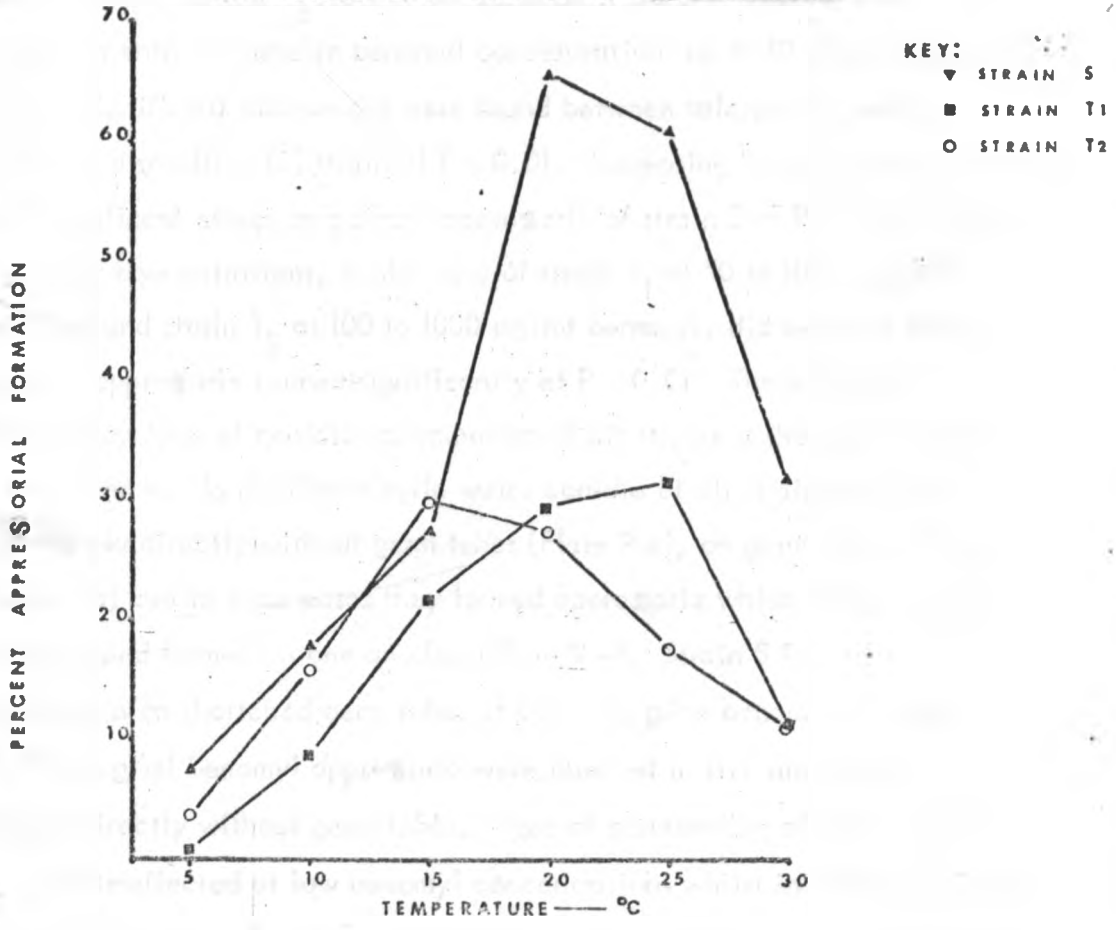
Table 9: Germination of C. coffeanum conidia harvested from sensitive (S) and tolerant (T_1 and T_2) strains grown on benomyl amended malt extract agar for one month

Benomyl conc. µg/ml	Strains - % germination S	T_1	T_2	Benomyl conc. means
0	58.9	25.0	21.4	35.1
0.25	37.5	26.1	21.4	28.3
0.5	33.9	26.0	23.3	27.7
1	21.6	26.6	21.9	23.4
2	1.6	25.1	22.7	16.5
2.5	0.0	26.5	22.7	16.4
5	0.0	26.2	23.5	16.6
10	0.0	25.5	23.1	16.2
25	0.0	25.2	22.2	15.8
50	0.0	25.0	21.5	15.5
100	0.0	22.7	20.9	14.5
125	0.0	22.6	21.0	14.6
250	0.0	22.7	12.8	11.8
500	0.0	21.8	7.7	9.8
1000	0.0	1.7	1.2	1.0
Strain means	10.2	23.3	19.2	

	5% level	1% level
L S D (Strains)	0.40	0.54
L S D (Concentrations)	0.90	1.20

total percent appressorial formation and type of conidial germination of the three strains. Summarised data is given in Fig 8 and appendix 14. At 5°C, germination of conidia of strain S was observed at 48 hr while germination for sirains T_1 and T_2 was observed at 72 hr. At 10, 15, 20, 25 and 30°C, conidial germination and appressorial formation of strain S were noted at 8 hr. These phenomena were varied for tolerant strains, T_1 and T_2. At 10 and 15°C strain T_1 showed conidial germination and appressorial formation at 24 hr while at 20, 25 and 30°C, conidial germination and appressorial formation were noted at 8 hr. Conidial germination and appressorial formation of strain T_2 were observed at 24 hr at 10, 15, 20, 25 and 30°C (appendix 14). Total percent appressorial formation varied according to temperature and strain at 72 hr. At 72 hr, each strain achieved a peak of percent appressorial formation at different temperatures. The highest percent appressorial formation for strain S was 65.3% at 20°C, strain T_1 was 31.3% at 25°C and strain T_2 was 29.9% at 15°C. The effect of temperature on type of germination of the three strains is shown in Plates 9 a - c and 9 e - f. At 5°C, all strains showed direct germination of conidia where no germ tube was formed but appressoria formed directly on conidia (Plate 9 c). At 10, 15, 20, 25 and 30°C, direct germination, germinated conidia with one germ tube with or without appressorium and germinated conidia with two germ tubes or more with or without appressoria were observed for all strains (Plates 9 c, a, and d). In some cases the germ tube bifurcated to form more than one appressorium (Plate 9 b). In rare cases the appressoria were bilobed, irregular in shape and formed other appressoria (Plate 9 e). However, at 30°C most of the germ tubes of the conidia of all strains were without appressoria and if appressoria formed they were on long germ tubes (Plate 9 f). There were significant differences between tolerant strains (T_1 and T_2) and sensitive strain (S) at $P = 0.01$. The effect of temperature on appressorial formation of all strains was significantly different at $P = 0.05$.

Fig. 8: THE EFFECT OF TEMPERATURE ON % APPRESSORIAL FORMATION OF STRAINS S, T1 AND T2 OF C. COFFEANUM AT 72 HR.



4.4.7. The effect of different benomyl concentrations on appressorial formation:

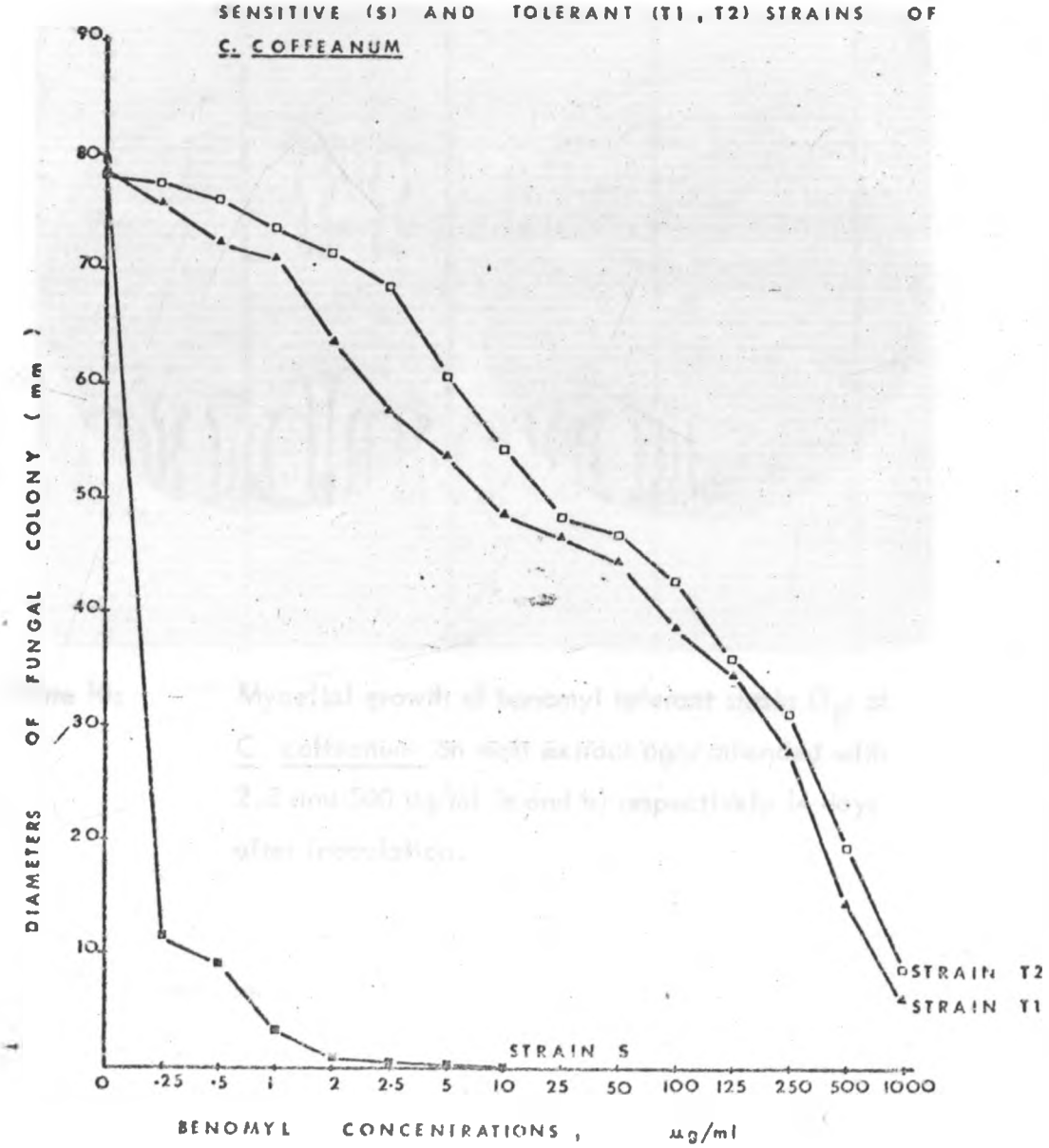
The effect of benomyl dilutions on percent appressorial formation of all strains is shown in Table 10. Strain S showed 64.3% appressorial formation at 0 µg/ml benomyl compared to 0.9% and 0% appressorial formation at 2.5 and 100 µg/ml benomyl respectively. Strain T_1 showed 27.2% appressorial formation at 0 µg/ml benomyl compared to 22.9% and 5.7% appressorial formation at 2.5 and 100 µg/ml benomyl respectively. Strain T_2 showed 19.3% in distilled sterile water compared to 22.9% and 16.2% appressorial formation at 2.5 and 100 µg/ml benomyl respectively. Strain T_2 showed an increase in percent appressorial formation with increase in benomyl concentrations up to 10 µg/ml benomyl. Highly significant differences were found between tolerant (T_1 and T_2) strains and sensitive (S) strain at $P = 0.01$. Increasing benomyl concentrations had significant effect on percent appressoria of strain S at $P = 0.01$. Only at higher concentrations, in the case of strain T_1 at 50 to 1000 µg/ml benomyl and strain T_2 at 100 to 1000 µg/ml benomyl, did benomyl affect percent appressoria formed significantly at $P = 0.01$. The effect of benomyl on type of conidial germination of all strains is shown in Plates 9 a - c and e. In distilled sterile water conidia of all strains produced appressoria directly without germ tubes (Plate 9 c), on germ tubes (Plates 9 a and b) and in some cases they formed appressoria which were irregular in shape and formed on one another (Plate 9 e). Strain S formed appressoria on shortened germ tubes at 0.5 - 1 µg/ml benomyl while at 2 - 2.5 µg/ml benomyl appressoria were reduced in size and mainly formed directly without germ tubes. Type of germination of strains T_1 and T_2 were unaffected at low benomyl concentrations whilst in 100 or 125 µg/ml benomyl solutions appressoria formed on shortened germ tubes. At 250 µg/ml benomyl, small appressoria of strains T_1 and T_2 mostly formed directly without germ tubes.

Table 10: The effect of benomyl dilutions on % appressorial formation of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Benomyl conc. µg/ml	STRAINS S	T_1	T_2	Benomyl conc. means
0	64.33	27.23	19.30	36.95
0.25	17.53	28.39	21.99	22.64
0.5	7.87	29.42	22.18	19.83
1	5.91	26.72	24.83	19.16
2	3.76	23.46	21.49	16.24
2.5	0.89	22.94	22.87	15.57
5	0.51	21.18	22.68	14.79
10	0.0	21.71	21.26	14.33
25	0.0	19.28	18.46	12.58
50	0.0	16.20	19.77	11.99
100	0.0	5.73	16.21	7.32
125	0.0	2.77	7.65	3.47
250	0.0	0.0	2.14	0.71
500	0.0	0.0	0.0	0.0
1000	0.0	0.0	0.0	0.0
Strain means	6.72	16.34	16.06	

	5% level	1% level
L S D (benomyl concs)	0.95	1.26
L S D (strains)	0.42	0.56

Fig. 9: THE EFFECT OF BENOMYL ON MYCELIAL GROWTH OF SENSITIVE (S) AND TOLERANT (T1, T2) STRAINS OF C. COFFEANUM



a b

Plate 10: Mycelial growth of benomyl tolerant strain (T_1) of C. coffeanum on malt extract agar amended with 2.5 and 500 µg/ml (a and b) respectively 14 days after inoculation.

4.5. The effect of different benomyl concentrations on mycelial growth, sporulating capacity and conidial size of benomyl sensitive and tolerant strains:

4.5.1. Mycelial growth:

The effect of benomyl on mycelial growth of the three strains is given in Fig 9. No considerable variation in colony diameter was apparent in both sensitive and tolerant strains on MEA without benomyl. However, mycelial growth of strain S was strongly inhibited by benomyl concentrations of 1 µg/ml and higher. Where little or no growth was seen after two weeks the inoculum discs were transferred to fresh plates of the medium and re-incubated for a further two weeks to determine whether the treatments were fungistatic or fungicidal. Growth of strains T_1 and T_2 was not greatly affected on MEA amended with benomyl up to 100 µg/ml but was greatly reduced on MEA amended with 500 and 1000 µg/ml benomyl (Fig 9 and Plate 10). In rare cases very light pink to pink and whitish sectors of growing cultures on MEA were observed (Plate 7). Occasionally distinct zonations occurred on mycelial mats (Plate 8). The differences among strains and also benomyl concentrations were highly significant at P = 0.001 as shown in appendix 15. Benomyl concentrations had great influence on radial mycelial growth of the strains and this interaction was highly significant at P = 0.001.

The re-incubation of strain S inoculum discs, from MEA with increasing concentrations of benomyl, after two weeks indicated that benomyl could either be fungistatic or fungicidal in action on mycelia depending on concentration. Benomyl concentrations of 0.25 to 500 µg/ml were fungistatic. However, radial mycelial growth of strain S was gradually reduced with increasing concentrations from 10 to 500 µg/ml benomyl. At 1000 µg/ml concentration, benomyl was fungicidal on mycelia and inoculum discs from MEA amended with this concentration had no growth on non-amended MEA when re-incubated.

4.5.2. Sporulating capacity:

The effect of benomyl on sporulation of all strains is given in

Table 11: The effect of benomyl on sporulating capacity of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum

Benomyl conc. µg/ml	Strain conidial count (x 1000)/mm^2			Benomyl conc. means
	S	T_1	T_2	
0	25.4	31.5	32.7	29.8
0.25	8.6	31.3	32.4	24.1
0.5	4.5	32.0	32.5	23.0
1	2.7	31.4	32.6	22.2
2	2.9	31.3	32.0	21.1
2.5	2.8	31.4	31.8	21.1
5	0.0	31.8	31.8	21.2
10	0.0	32.3	33.0	21.7
25	0.0	32.3	39.4	23.9
50	0.0	43.7	43.4	29.0
100	0.0	45.4	48.9	31.4
125	0.0	42.4	54.0	32.1
250	0.0	40.9	40.8	27.3
500	0.0	29.0	34.0	21.0
1000	0.0	14.8	32.8	15.9
Strain	3.1	33.4	36.8	

	5% level	1% level
L S D (Concentrations)	1.2	1.6
L S D (Strains)	0.5	0.7

Table II. Initially when conidia were used in sporulation experiment no colonies grew on MEA with 500 and 1000 µg/ml benomyl. The method of inoculating MEA plates, with increasing concentrations of benomyl, was changed to use of mycelial discs of a given strain. Sporulation of sensitive and tolerant strains differed by a magnitude of approximately 6,000 conidia/mm^2 after 21 days of incubation on MEA plates without benomyl. Strain S produced 25.4×10^3 conidia /mm^2 compared to 31.5×10^3 and 32.7×10^3 conidia/mm^2 produced by strains T_1 and T_2 respectively in MEA plates without benomyl. There was a stimulation of conidial production up to 250 µg/ml benomyl for strain T_1 and up to 500 µg/ml benomyl for strain T_2 as shown in Table II. Increasing benomyl concentration greatly reduced conidial production of strain S. There were significant differences between sensitive (S) strain and tolerant (T_1 and T_2) strains and also among benomyl concentrations at P = 0.01. Sectoring and zonation was occasionally observed.

4.5.3. Conidial size:

Microscopic observation showed that the size of conidia was not reduced when strains T_1 and T_2 were grown on MEA amended with increasing concentrations of benomyl (Table 12). Strain S did not grow on MEA containing 5 µg/ml benomyl and thus it was not possible to obtain any conidia from MEA with higher benomyl concentrations to measure conidial size. The conidial size for all strains from non-amended MEA ranged from 9.78 to 22.82 by 3.26 to 9.78µ. This variation in conidial size was observed in all benomyl concentrations for strains T_1 and T_2 and in low benomyl concentrations for strain S.

4.6. The effect of subculturing benomyl tolerant strains:

4.6.1. Mycelial growth:

Table 13 gives a summary of the results of radial mycelial growth

Table 12: The effect of benomyl on conidial size (μ) of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Benomyl Conc. μg/ml	Strains S W	S L	T_1 W	T_1 L	T_2 W	T_2 L	Benomyl conc. means W	Benomyl conc. means L
0	5.7	15.9	5.3	16.3	5.3	15.1	5.4	15.8
0.25	5.7	17.1	4.9	15.5	6.1	14.3	5.6	15.6
0.5	5.3	15.9	5.3	15.9	5.3	15.5	5.3	15.8
1	5.5	16.3	4.9	15.1	5.3	15.1	5.6	15.5
2	5.3	14.8	7.3	15.1	6.1	14.5	6.3	14.8
2.5	6.0	15.8	5.7	17.1	5.7	16.3	5.8	16.4
5	0.0	0.0	5.3	15.1	6.1	13.3	3.8	9.5
10	0.0	0.0	5.7	16.7	6.9	17.1	4.2	11.3
25	0.0	0.0	5.7	14.7	6.5	14.7	4.1	9.8
50	0.0	0.0	6.5	15.5	6.5	16.7	4.3	10.7
100	0.0	0.0	5.3	15.5	5.7	16.7	3.7	10.7
125	0.0	0.0	5.7	15.5	6.9	17.5	4.2	11.0
250	0.0	0.0	5.3	15.9	5.7	15.5	3.7	10.5
500	0.0	0.0	6.0	16.0	5.2	15.6	3.7	10.6
1000	0.0	0.0	5.6	15.4	5.8	15.4	3.8	10.2
Strain means	2.3	6.4	5.6	15.7	6.0	15.5		

Note: W - width: L - Length

		5% level	1% level
Width:	L S D (strains)	0.38	0.50
	L S D (benomyl conc.)	0.84	1.11
Length:	L S D (strains)	0.64	0.84
	L S D (benomyl conc.)	1.43	1.89

Table 13: The effect of a series of subculturing on mycelial growth (mm) of benomyl sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Strain (1)	Subculturing	Benomyl concentrations - µg/ml (F.C.) 0.0	0.25	0.5	2.5	5	10	100	125	250	1000	1xsubc Means	Strain Means
S	Subc.1	80.17	11.33	5.00	0.72	0.0	0.0	0.0	0.0	0.0	0.0	9.72	
	Subc.2	79.50	11.83	5.67	0.75	0.0	0.0	0.0	0.0	0.0	0.0	9.78	
	Means (1xF.C.)	79.83	11.58	5.33	0.73	0.0	0.0	0.0	0.0	0.0	0.0		9.75
T_1	Subc.1	77.00	78.33	78.33	79.17	79.67	80.83	31.17	31.17	22.67	14.83	57.32	
	Subc.2	77.67	79.00	79.33	79.67	79.00	83.33	30.83	30.16	23.33	15.67	57.92	
	Means (1xF.C.)	77.34	78.67	78.83	79.42	79.33	82.08	31.00	30.67	23.00	15.25		57.62
T_2	Subc.1	79.00	79.50	78.83	79.33	81.17	79.17	32.00	28.00	22.33	21.50	58.08	
	Subc.2	79.67	78.83	80.67	79.33	81.17	77.17	32.00	25.67	23.17	22.50	57.98	
	Means (1xF.C.)	79.33	79.17	79.75	79.33	81.17	78.17	32.00	26.83	22.75	22.00		58.03
													Means (Subc.)
Means (F.C.xSubc.)	Subc.1	78.72	56.39	54.06	53.07	53.61	53.33	21.06	19.72	15.00	12.11		41.71
	Subc.2	79.28	56.56	55.22	53.25	53.39	53.50	20.94	18.61	51.50	12.72		41.89
	Means (F.C.)	79.00	56.47	54.64	53.16	53.50	53.42	21.00	19.17	15.25	12.42		

	5% level	1% level
L S D (Strains)	0.45	0.60
L S D (Fungicide conc.)	0.82	1.09
L S D (Subculturing)	0.37	0.49
L S D (Subc. x 1)	0.64	0.84
L S D (Subc. x F.C.)	1.16	1.54

of the three strains, on benomyl amended MEA, after several transfers on non-amended MEA. Tolerance of strains T_1 and T_2 was not reduced by prolonged growth on non-amended MEA. Subculturing had no significant effect at P = 0.05 on the response of the strains to benomyl. Strain S had very limited radial mycelial growth of 0.72 mm and 0.75 mm, at 2.5 µg/ml benomyl, when subcultured once and 12 times on MEA respectively. Strain T_1 had radial mycelial growth measuring 31.17 mm and 30.83 mm, at 100 µg/ml benomyl, when subcultured once and 12 times on MEA respectively. Strain T_2 had radial mycelial growth measuring 32 mm, at 100 µg/ml benomyl, when subcultured both once and 12 times on MEA. According to these observations, benomyl tolerant strains, T_1 and T_2, retained their tolerance to benomyl at the same level for six months after 12 transfers on benomyl free MEA. There were significant differences between tolerant strains and sensitive strain and also among benomyl concentrations at P = 0.01.

4.6.2. Sporulating capacity:

Table 14 is a summary of the results of sporulating capacity of the three strains. Although strains T_1 and T_2 grew at high concentration of benomyl, the sporulation phenomenon was affected by increasing concentration of benomyl. From 10 to 250 µg/ml benomyl, conidial production was stimulated and then showed a decline at 1000 µg/ml benomyl. In all the three strains, conidial production was low for all the repeatedly subcultured "isolates" on fungicide free MEA. There were significant differences among the strains and between subculturing at P = 0.01. The interaction between subculturing and strains was highly significant at P = 0.01.

4.6.3. Conidial germination:

Table 15 gives a summary of the results of appressorial formation of the three strains in several benomyl dilutions. Strains T_1 and T_2 were

Table 14: The effect of a series of subculturing on sporulating capacity of benomyl sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (conidia/mm^2 x 1000)

Strain (1)	Subculturing (Subc.)	Benomyl concentrations (F.C.) - µg/ml 0	.25	.5	2.5	5	10	100	125	250	1000	Means 1xSubc.	Means strain
S	Subc.1	25.4	8.4	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	
	Subc. 2	25.2	8.1	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	
	Means (1xF.c.)	25.3	8.2	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0		4.0
T_1	Subc.1	38.0	37.3	37.4	37.7	38.2	41.6	58.7	63.0	69.1	25.0	42.6	
	Subc.2	35.8	36.1	36.1	36.4	36.3	37.3	42.4	44.3	35.4	13.8	35.4	
	Means (1xF.C.)	36.9	36.7	36.8	37.0	37.3	39.5	50.5	53.7	42.2	19.4		39.0
T_2	Subc.1	38.2	38.2	37.8	36.8	37.5	44.5	67.6	57.2	47.7	35.4	44.1	
	Subc.2	35.0	36.8	35.6	35.6	35.7	35.4	65.3	48.8	42.0	28.4	39.8	
	Means (1xF.C.)	36.6	37.5	36.7	36.2	36.6	39.9	66.5	53.0	44.9	31.9		42.0
													Means (Subc.)
Means (F.C. x Subc.)	Subc.1	33.9	28.0	27.7	24.8	25.2	28.7	42.1	40.1	32.3	20.1		30.3
	Subc.2	32.0	27.0	25.3	24.0	24.0	24.2	35.9	31.0	25.8	14.1		26.3
	Means (F.C.)	32.9	27.5	26.5	24.4	24.6	26.5	39.0	35.5	29.0	17.1		

	5% level	1% level
L S D (Strains)	0.18	0.23
L S D (Fungicide conc.)	0.32	0.42
L S D (Subculturing)	0.14	0.19
L S D (Subc. x 1)	0.25	0.33
L S D (Subc. x F.C.)	0.45	0.60

Table 15: The effect of a series of subculturing on conidial germination and appressorial formation of benomyl sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (% appressorial formation)

Strain (1)	Subculturing (Subc.)	Benomyl concentrations (F.C) - µg/ml 0	.25	.5	2.5	5	10	100	125	250	1000	Means 1 x Subc.	Means Strain
S	Subc.1	74.0	18.2	9.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	10.3	
	Subc.2	58.1	8.0	6.4	2.1	0.0	0.0	0.0	0.0	0.0	0.0	7.5	
	Means (1xF.C.)	66.1	13.1	7.8	1.5	0.0	0.0	0.0	0.0	0.0	0.0		8.9
T_1	Subc.1	30.5	30.1	30.5	21.6	21.4	21.2	7.2	4.7	1.7	0.0	16.8	
	Subc.2	20.2	20.4	20.9	23.4	22.6	21.8	10.8	8.3	2.9	0.0	15.1	
	Means (1xF.C.)	25.4	25.2	25.7	22.5	22.0	21.0	9.0	6.5	2.3	0.0		16.0
T_2	Subc.1	23.2	23.2	22.9	22.4	20.4	18.1	9.8	6.5	1.5	0.0	14.8	
	Subc.2	18.5	19.5	19.9	18.9	18.3	18.4	11.7	9.3	1.3	0.0	13.6	
	Means (1xF.C.)	20.9	21.3	21.4	20.6	19.3	18.3	10.7	7.9	1.4	0.0		14.2
													Means (Subc.)
Means (F.C.xSubc.)	Subc.1	42.6	23.8	20.9	15.0	13.9	12.8	5.7	3.8	1.1	0.0		13.9
	Subc.2	32.3	16.0	15.7	14.8	13.6	13.4	7.5	5.9	1.4	0.0		12.1
	Means (F.C.)	37.4	19.9	18.3	14.9	13.8	13.1	6.6	4.8	1.2	0.0		

	5% level	1% level
L S D (Strains)	0.22	0.29
L S D (Fungicide conc.)	0.41	0.54
L S D (Subculturing)	0.18	0.24
L S D (Subc. x 1)	0.31	0.42
L S D (Subc. x F.C.)	0.57	0.76

subcultured on fresh fungicide free MEA about twice each month for six months without loss of tolerance. Conidia of strains T_1 and T_2 were able to form appressoria in up to 250 µg/ml benomyl concentration while conidia of strain S formed appressoria in up to 2.5 µg/ml benomyl concentration. The formation of appressoria was lower for all the repeatedly subcultured "isolates" on fungicide free MEA compared to the "isolates" subcultured once on fungicide free MEA at lower benomyl concentration. However, as benomyl concentration increased the repeated subcultured "isolates" formed high numbers of appressoria. As reported earlier on germination, most conidia of the three strains became uniseptate and one or two germ tubes were formed. In distilled sterile water, it was noted that appressoria formed on long germ tubes in the case of all repeatedly subcultured "isolates" compared to all "isolates" subcultured only once. This phenomenon was observed in strains T_1 and T_2 in the lower benomyl concentrations. Appressoria of strain S formed on shortened germ tubes at 0.5 to 1 µg/ml benomyl while at 2 to 2.5 µg/ml benomyl appressoria were reduced in size and formed mainly directly without germ tubes. At 250 µg/ml benomyl appressoria of strains T_1 and T_2, repeatedly subcultured and subcultured once, mostly formed directly without germ tubes and with their sizes reduced. There were significant differences among the strains and interaction between subculturing and strains at $P = 0.01$. There were significant differences among the fungicide concentrations and also between subculturing at $P = 0.01$.

4.6.4. Berry infection:

All the benomyl tolerant strains, repeatedly subcultured and subcultured once on fungicide free MEA, were pathogenic as indicated by the development of typical sporulating lesions similar to those of the sensitive strain on detached berries of SL 28 (Plate I). Table 16 gives a summary of the results of percent berry infection of detached berries of SL 28 inoculated with "isolates" of C. coffeanum. The differences between strains, time of recording infection and subculturing were highly

Table 16: Pathogenicity of benomyl tolerant and sensitive strains of C. coffeanum repeatedly subcultured for 6 months on fungicide free M.A:

Table of means (% berry infection)

Strains (1)	Subculturing Subc.	Time of recording infection (D) D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	1 x Subc.	Strain (Means)
S	S (Subc 1)	0	9.3	45.3	49.3	73.3	74.7	78.7	81.3	51.5	
	SB (Subc 2)	0	16.0	70.7	81.3	90.7	90.7	90.7	90.7	66.3	
	1 x D	0	12.7	58.0	65.3	82.0	82.7	84.7	86.0		58.9
T_1	T_1 (Subc.1)	0	20.0	74.7	80.0	86.7	86.7	86.7	86.7	65.2	
	T_1B (Subc 2)	0	44.0	85.3	90.7	92.0	94.7	96.0	96.0	74.8	
	I x D	0	32.0	80.0	85.3	89.3	90.7	91.3	91.3		70.0
T_2	T_2 (Subc 1)	0	16.0	44.0	58.7	73.3	77.3	80.0	81.3	53.8	
	T_2B (Subc 2)	0	29.3	68.0	74.7	89.3	90.7	94.7	94.7	67.7	
	1 x D	0	22.7	56.0	66.7	81.3	84.0	87.3	88.0		60.8
											Subc (Mean)
	Sub 1 x D	0	15.1	54.7	62.7	77.8	79.6	81.8	83.1		56.8
	Subc 2 x D	0	29.8	74.7	82.2	90.7	92.0	93.8	93.8		69.6
	D(Mean)	0	22.4	64.7	72.4	84.2	85.8	87.8	88.4		

	5% level	1% level
L S D (Strains)	3.0	3.9
L S D (D)	4.9	6.4
L S D (1 x D)	8.4	11.1
L S D (Subc.)	2.4	3.2
L S D (Subc x 1)	4.2	5.6
L S D (Subc x D)	6.9	9.1

significant at P = 0.01. The interactions between subculturing and strains and subculturing and time of recording infection were also highly significant at P = 0.01.

4.7. Anastomosis:

4.7.1. Conidial germination:

Germination of the conidia started by forming a clear pseudoseptum in the centre. Germ tube fusion was noted for all the three strains although it was not very frequent. A few chlamydospores formed after the germ tube extension stopped. However, as the hyphae started branching out hyphal fusion was noted. It was observed that conidia of benomyl tolerant strains showed slight delay in germination compared to conidia of benomyl sensitive strain. Conidial production was noted on the fourth day of incubation for the benomyl sensitive strain on plain agar slide (Plate 11).

4.7.2. Hyphal growth:

After 14 days of incubation, hyphal fusion was well distributed within the mycelia of all the three strains and conidia were borne singly on conidiophores (Plates 13 and 11). The condiophores were more heavily stained as compared to the vegetative hyphae. All the strains had many chlamydospores formed both terminally and intercalarily (Plates 12 a and b).

4.8. Cross-tolerance:

4.8.1. Mycelial growth:

From the results shown in Table 17, it appeared that the benomyl tolerant strains were also tolerant to other benzimidazole fungicides, Derosal and Bavistin. Mycelial growth of strains T_1 and T_2 was less

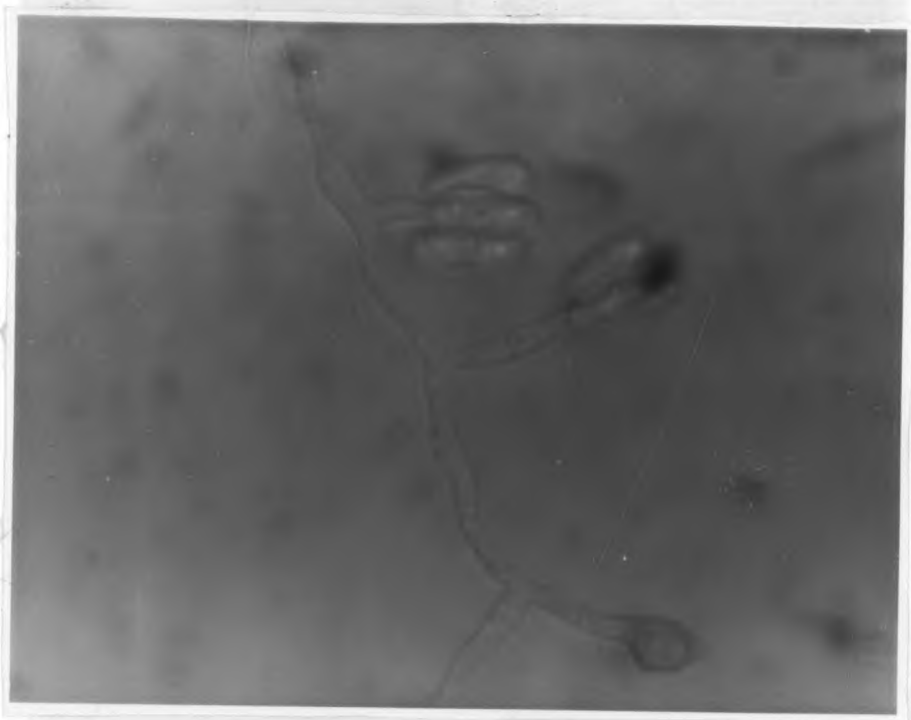


Plate II: Conidial production of strain S of C. coffeanum .

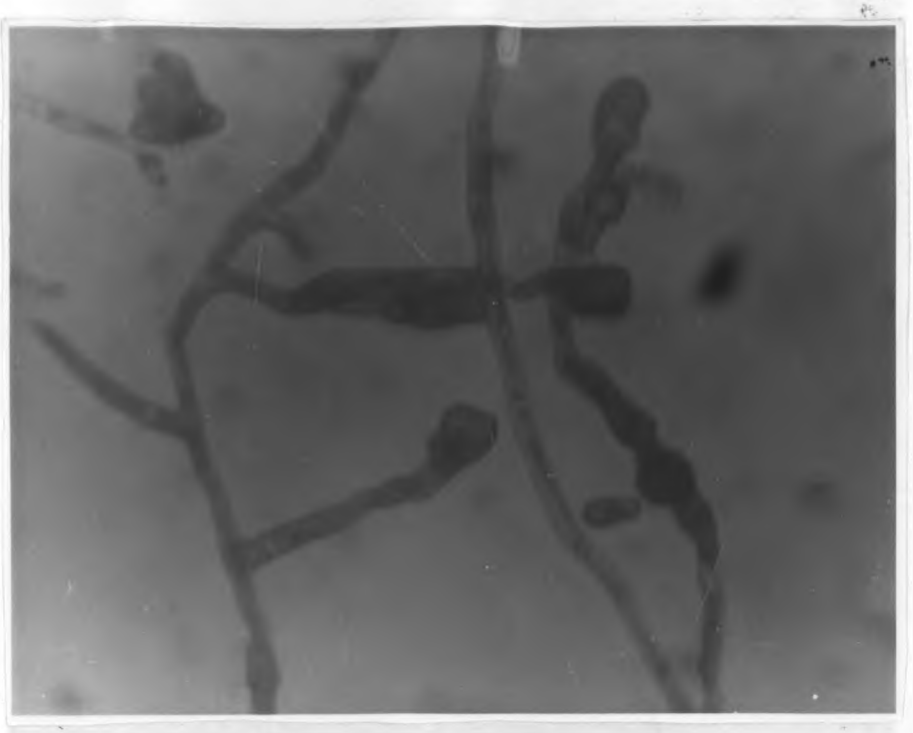


Plate 12 a: Formation of terminal chlamydospores of strain T_2 of C. coffeanum.

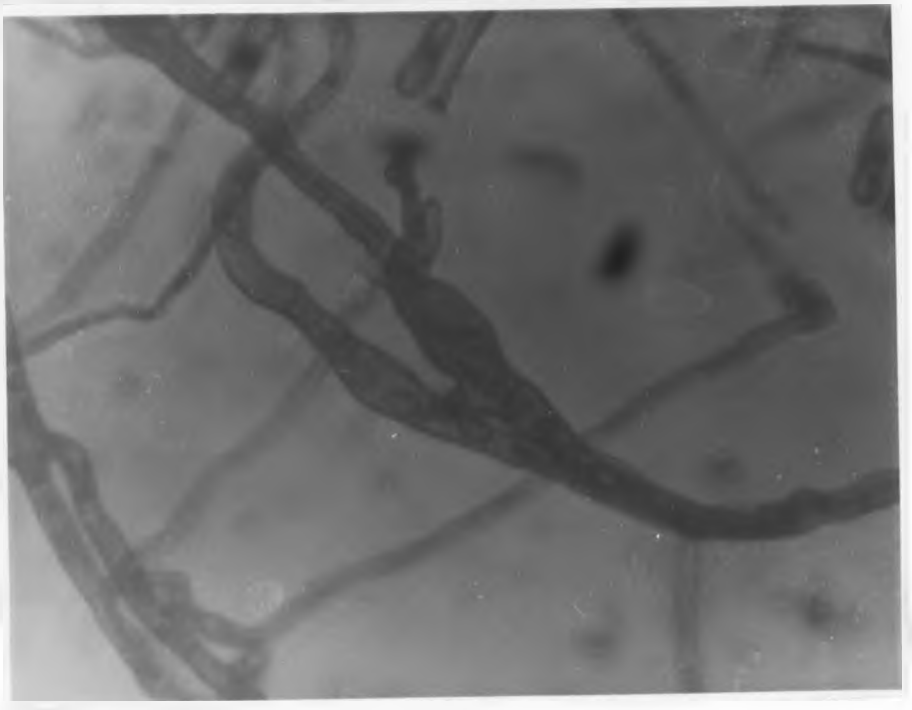


Plate 12 b: Formation of intercalary chlamydospores of strain T_2 of C. coffeanum.

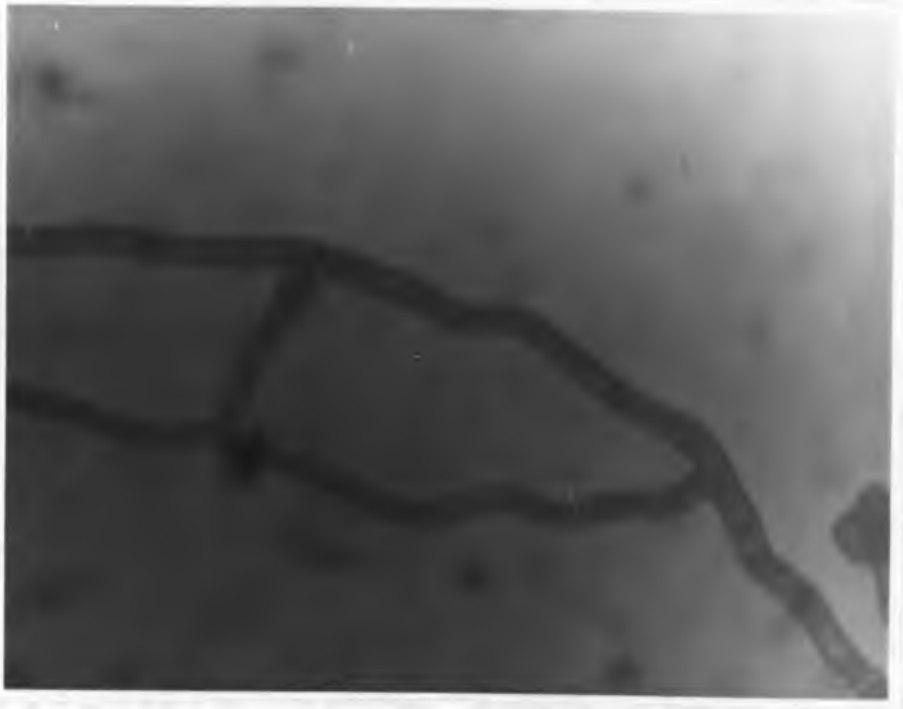


Plate 13: Hyphal fusion of strain T_1 of C. coffeanum

Table 17: Cross-tolerance: Mycelial growth (mm) of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Strain (I)	Fungicide (F)	Fungicide concentrations (F.C) - µg/ml 0	1	10	100	250	1000	Means (IxF)	Means (strains)
S	Benlate	77.33	2.67	0.00	0.00	0.00	0.00	13.33	
	Bavistin	76.67	2.83	0.00	0.00	0.00	0.00	13.25	
	Derosal	76.83	1.17	0.00	0.00	0.00	0.00	13.00	
Means									13.19
I x F.C		76.94	2.22	0.00	0.00	0.00	0:00		
T_1	Benlate	79.50	64.83	58.33	34.33	20.00	5.00	43.67	
	Bavistin	79.33	72.17	56.83	44.17	38.50	25.67	52.78	
	Derosal	79.17	77.50	63.50	44.33	36.17	24.83	54.25	
Means									50.23
I x F.C		79.33	71.50	59.56	40.94	31.56	18.50		
T_2	Benlate	79.17	61.00	50.83	34.00	23.50	13.33	43.64	
	Bavistin	79.17	70.50	58.00	45.83	36.33	30.67	53.42	
	Derosal	78.83	77.17	59.17	46.00	37.17	30.00	54.72	
Means									50.59
I x F.C		79.06	69.56	56.00	41.94	32.33	24.67		Means (F.)
Means	Benlate	78.67	42.83	36.39	22.78	14.50	6.11		33.55
F.C. x F	Bavistin	78.39	48.50	38.28	30.00	24.94	18.78		39.82
	Derosal	78.28	51.94	40.89	30.11	24.44	18.28		40.66
Means F.C.		78.44	47.76	38.52	27.63	21.30	14.39		

	5% level	1% level
L S D (Strains)	0.55	0.73
L S D (Fungicides)	0.55	0.73
L S D (Fungicide Conc.)	0.78	1.03
L S D (I x F)	0.95	1.26
L S D (I x F.C)	1.35	1.78
L S D (F x F.C)	1.35	1.78

inhibited on MEA with 1000 µg/ml a.i. Derosal or Bavistin than on MEA with 1000 g/ml benomyl after 14 days of incubation. Strain T_1 showed 5 mm in MEA amended with Benlate compared to 25.67 mm and 24.83 mm in MEA amended with Bavistin and Derosal respectively at the same concentration of 1000 µg/ml a.i. Strain T_2 had 13.33 mm in MEA with Benlate compared to 30.67 mm and 30 mm in MEA with Bavistin and Derosal respectively at similar concentration of 1000 µg/ml a.i. Strains T_1 and T_2 were even less inhibited when growing in MEA with 1000 µg/ml a.i. Derosal or Bavistin than strain S in MEA with 1 µg/ml a.i. Derosal or Bavistin after 14 days incubation. However, mycelial growth in the presence of benzimidazole fungicide declined gradually as concentrations were increased. There were significant differences between sensitive strain, S, and tolerant strains, T_1 and T_2, at $P = 0.01$. There were significant differences at $P = 0.01$ between benomyl and other benzimidazole fungicides, Derosal and Bavistin. The three fungicides did not influence mycelial growth of strain S differently. However, with increasing concentrations, benomyl greatly reduced radial mycelial growth of strains T_1 and T_2 compared to Derosal and Bavisitin and these differences were highly significant at $P = 0.01$. Occasionally sectoring and zonation of growing cultures in MEA was noted.

4.8.2. Sporulation:

The effect of benzimidazole fungicides on sporulation of the three strains is given in Table 18. Strain T_2 showed a higher level of sporulation ability in Derosal and Bavistin than in Benlate amended MEA with increasing fungicide concentrations from 10 to 1000 µg/ml a.i. Tolerance in conidial production to Derosal attained a higher level in strain T_1 than to Benlate and Bavistin as shown in Table 18. Conidial production of strain S was also sensitive to Derosal and Bavistin and no sporulation was recorded in MEA amended with 10 µg/ml a.i. of the benzimidazole fungicides. Sporulation of strains T_1 and T_2 was, generally, not adversely affected by increasing concentrations of Derosal or

Table 18: Cross-tolerance : Sporulating capacity of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (conidia/mm^2 x 1000)

Strain (1)	Fungicide (F)	Fungicide concentrations (F.C. - µg/ml) 0	1	10	100	250	1000	Means(1xF)	Means(strains)
S	Benlate	25.35	2.68	0.0	0.0	0.0	0.0	4.67	
	Bavistin	24.98	3.31	0.0	0.0	0.0	0.0	4.72	
	Derosal	25.30	5.15	0.0	0.0	0.0	0.0	5.07	
Means 1 x F.C		25.21	3.71	0.0	0.0	0.0	0.0		4.82
T_1	Benlate	31.87	31.35	36.65	54.53	46.68	17.83	36.49	
	Bavistin	31.84	32.13	44.87	50.44	44.76	26.61	38.44	
	Derosal	31.64	31.48	45.34	70.09	56.41	55.96	48.49	
Means 1 x F.C		31.78	31.65	42.29	58.35	49.28	33.47		41.14
T_2	Benlate	32.11	32.37	33.81	60.70	39.75	29.88	38.10	
	Bavistin	31.74	32.68	38.42	67.81	53.93	55.84	46.74	
	Derosal	32.24	45.54	66.27	79.22	77.14	56.47	59.48	
Means 1 x F.C		32.03	36.86	46.17	69.25	56.94	47.40		46.11 Means(F.)
Means F.C. x F.	Benlate	29.78	22.13	23.49	38.41	28.81	15.90		26.42
	Bavistin	29.52	22.71	27.77	39.42	32.90	27.48		29.97
	Derosal	29.72	27.39	37.20	49.77	44.52	37.48		37.68
Means F.C		29.67	24.08	29.49	42.53	35.41	26.96		

	5% level	1% level
L S D (Strains)	0.49	0.65
L S D (Fungicides)	0.49	0.65
L S D (Fungicide Conc.)	0.69	0.91
L S D (1 x F)	0.85	1.12
L S D (1 x F.C.)	1.20	1.58
L S D (F.x F.C.)	1.20	1.58

Bavistin as shown in Table 18. There were significant differences among the strains and also among fungicides at P = 0.01. The three fungicides did not affect sporulation of strain S differently. However, the three fungicides showed varied influence on sporulation of strains T_1 and T_2 and these differences were highly significant at P = 0.01. In rare cases sectoring and zonation of growing cultures in MEA was observed.

4.9. Competitive behaviour of benomyl sensitive and tolerant strains:

Mixed inoculation tests of benomyl sensitive (S) and tolerant strains (T_1 and T_2) were carried out on detached berries. In re-isolations from 119 berries infected with a 1:1 mixture of S: T_1 only 14 berries (approx. 12%) yielded a pure benomyl tolerant population while 105 berries (approx. 88%) yielded a mixed population. A similar pattern was observed in berries inoculated with a mixture of strains S and T_2. Out of 112 berries, 14 berries (12.5%) yielded a pure benomyl tolerant population and 98 berries (87.5%) yielded a mixed population. Re-isolations made from berries inoculated with mixture of conidia from tolerant and sensitive strains did not yield pure benomyl sensitive population. Significant differences of strain mixtures at P = 0.001 could be due to differences in ratios of benomyl sensitive and tolerant strains re-isolated. However, the interaction between strain mixtures and batches was not significantly different at P = 0.05 (Table 19).

4.10. In vitro control of benomyl tolerant strains

4.10.1. Effect of fungicides and their combinations on conidial germination of benomyl tolerant and sensitive strains:

Conidial germination of the three strains in various dilutions of benomyl, captafol and Copper Nordox and their combinations is given in Fig 10 and Table 20. There were significant differences among strains

Table 19: Competitive bahaviour of benomyl tolerant and sensitive strains of C. coffeanum:

Table of means

Strain Mixture	Batch	Percent infection means
S: T_1	1	56.4
	2	79.6
	3	68.9
Mix x Batch Mean		68.3
S: T_2	1	78.1
	2	72.4
	3	66.4
Mix x Batch Means		72.3

	5% level	1% level
L S D (Mixture x Batch Means)	6.8	9.0

Fig.10: EFFECT OF BENOMYL AND OTHER FUNGICIDES ON CONIDIAL GERMINATION OF SENSITIVE (S) AND TOLERANT (T_1 & T_2) STRAINS OF C. COFFEANUM

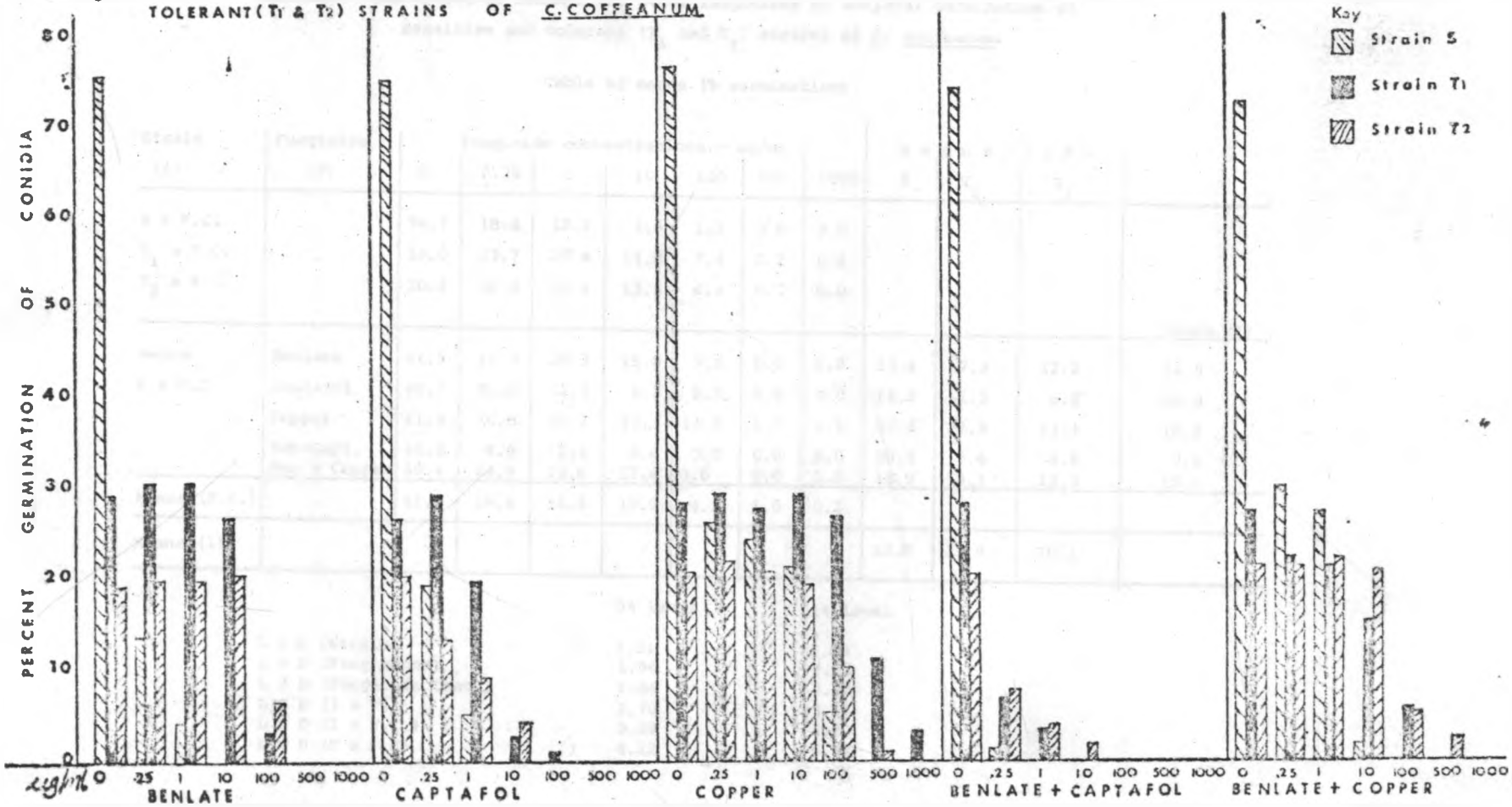


Table 20: The effect of benomyl and other fungicides on conidial germination of sensitive and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (% germination)

Strain (1)	Fungicide (F)	Fungicide concentrations - µg/ml 0	0.25	1	10	100	500	1000	Means (1 x F) S	T_1	T_2	Means (F)
S x F.C.		74.7	18.4	12.2	4.6	1.1	0.0	0.0				
T_1 x F.C.		28.0	23.7	20.4	14.8	7.4	2.2	0.6				
T_2 x F.C.		20.4	16.8	15.2	13.3	4.4	0.7	0.0				
Means	Benlate	41.5	21.3	18.3	15.8	3.3	0.0	0.0	13.4	17.3	12.2	14.3
F x F.C.	Captafol	40.7	20.8	11.3	2.3	0.3	0.0	0.0	14.2	11.3	6.8	10.8
	Copper	41.8	26.0	24.2	23.3	14.2	4.0	1.1	22.0	22.3	13.4	19.2
	Ben+Capt.	40.8	5.3	2.4	0.6	0.0	0.0	0.0	10.8	5.4	4.8	7.0
	Ben + Copper	40.4	24.3	23.6	12.6	3.6	0.8	0.0	18.9	13.1	13.3	15.1
Means (F.C.)		41.0	19.6	15.9	10.9	4.3	1.0	0.2				
Means (1)									15.9	13.9	10.1	

	5% level	1% level
L S D (Strains	1.21	1.59
L S D (Fungicides)	1.56	2.05
L S D (Fungicide Conc.)	1.84	2.42
L S D (1 x F)	2.70	3.55
L S D (1 x F.C.)	3,19	4.20
L S D (F x F.C)	4.12	5.42

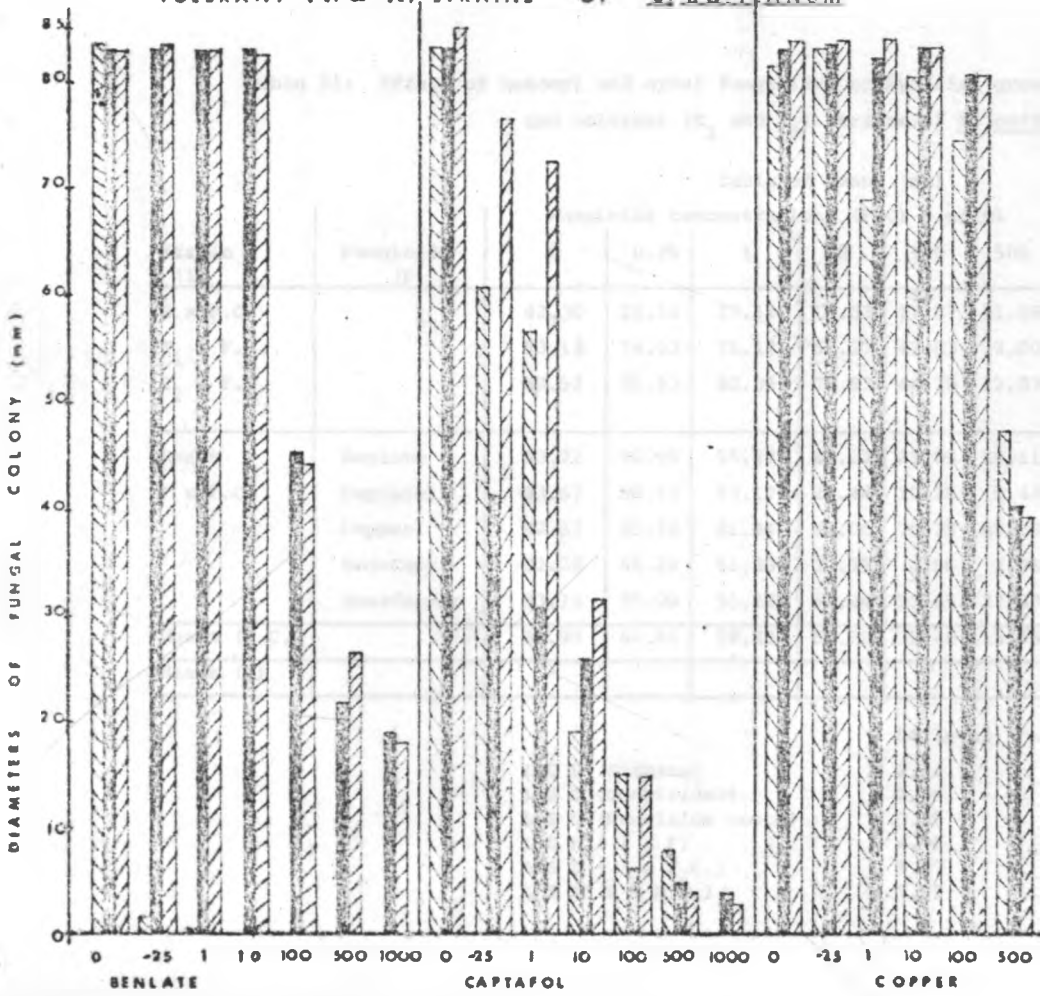
and also among fungicides with their combinations at P = 0.01. Significant differences were found between fungicide concentrations at lower levels, 0 - 100 concentrations, at P = 0.01 but not at 500 and 1000 µg/ml a.i. of fungicide as shown in Table 20. The interactions between strains and fungicide concentrations were found to be significantly different (P = 0.05) at 0 - 100 µg/ml concentration levels but no significant differences were found between 500 - 1000 µg/ml concentration levels. Benomyl and captafol at 10 µg/ml and above prevented conidial germination of strain S. Benomyl plus captafol and benomyl plus Copper Nordox at 1 and 100 µg/ml and above respectively prevented conidial germination while Copper Nordox at 500 and 1000 µg/ml prevented conidial germination of strain S. Concentrations of 0.25 µg/ml of fungicides did not affect type of germination of strain S and types of germination were as reported earlier in distilled sterile water. At 1 µg/ml benomyl, appressoria formed directly on conidia or on highly reduced germ tubes. At 1 µg/ml benomyl plus Copper Nordox, conidia of strain S formed long germ tubes with or without appressoria. At 10 µg/ml benomyl plus Copper Nordox, very few conidia formed long germ tubes without appressoria otherwise most germinated conidia had appressoria without germ tubes. At higher concentrations of Copper Nordox (10 - 100 µg/ml), conidia of strain S formed long germ tubes and only a few had appressoria. At concentrations of 1 µg/ml of fungicides and below, conidial germination of strains T_1 and T_2 was not adversely affected and types of conidial germination reported earlier were observed. At 10 µg/ml of benomyl, Copper Nordox and benomyl plus Copper Nordox did not inhibit conidial germination of strains T_1 and T_2. However, at concentration of 100 µg/ml and above percent conidial germination became reduced considerably. Percent conidial germination of strains T_1 and T_2 decreased sharply with increasing concentrations of captafol and benomyl plus captafol. At 100 to 500 µg/ml Copper Nordox, conidia of strains T_1 and T_2 formed long germ tubes with just a few of them having appressoria while at 1000 µg/ml, conidia of strain T_1 had shortened germ tubes and a few of them had appressoria. This experiment did indicate that conidial

germination of strains T_1 and T_2 was effectively inhibited by benomyl plus captafol at 100 µg/ml concentration followed by captafol alone at 100 µg/ml concentration.

4.10.2 Mycelial growth:

Summaries of the results of radial mycelial growth of the three strains in MEA amended with benomyl, captafol and Copper Nordox and their combinations are given in Fig 11 and Table 21. Differences among the strains, fungicides and fungicide concentrations were highly significant at $P = 0.001$. Interactions between strains and fungicides and between strains and fungicide concentrations were highly significant at $P = 0.001$. The interactions between fungicides and fungicide concentrations were significantly different at $P = 0.01$. In all cases third order interaction was used to test their differences. However, benomyl, benomyl plus captafol and benomyl plus Copper Nordox had great effect on radial mycelial growth of strain S and the interactions were not significantly different from one another at $P = 0.05$ (Table 21). Mycelial growth of strain S was still strongly inhibited by benomyl up to 1 µg/ml and no mycelial growth occurred at 10 µg/ml benomyl. At very low concentrations of 0.25 µg/ml of combinations of benomyl plus captafol and benomyl plus Copper Nordox, mycelial growth of strain S was strongly inhibited. Mycelial growth in the presence of captafol and Copper Nordox declined more rapidly and gradually as concentrations were increased respectively. However, neither fungicide was as active as benomyl at low concentrations against strain S. Strains T_1 and T_2 were able to grow on MEA amended with captafol and Copper Nordox and their combinations although differences in radial mycelial growth according to fungicide concentrations were found. Radial mycelial growth of strains T_1 and T_2 on MEA amended with captafol decreased sharply between 0.25 and 10 µg/ml and was highly suppressed at 100 to 1000 µg/ml captafol and benomyl plus captafol. Radial mycelial growth of strains T_1 and T_2 on MEA amended with Copper Nordox, benomyl and their combination declined more gradually as

Fig.11: THE EFFECT OF BENOMYL AND OTHER FUNGICIDES ON TOLERANT (T_1 & T_2) STRAINS OF C. COFFEANUM



MYCELIAL GROWTH OF SENSITIVE (S) AND

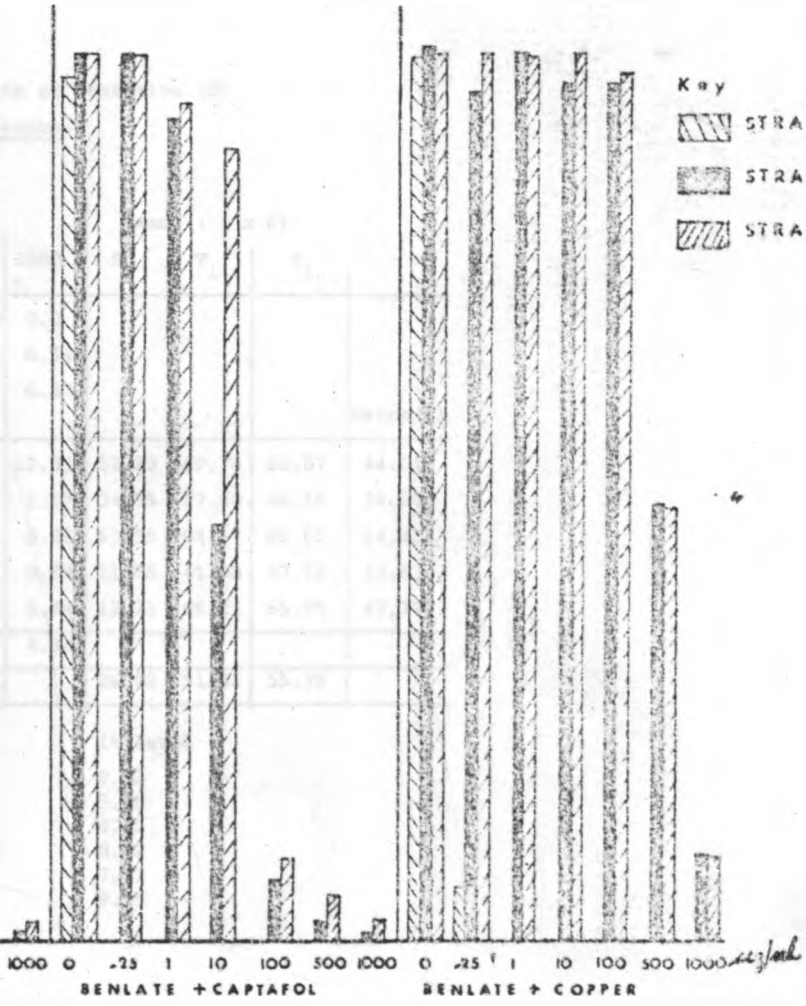


Table 21: Effect of benomyl and other fungicides on mycelial growth of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (mm)

Strain (1)	Fungicide (F)	Fungicide concentrations (F.C) - µg/ml 0	0.25	1	10	100	500	1000	Means (1 x F) S	T_1	T_1	Means (F)
S x F.C.		82.30	29.56	27.12	19.83	17.87	11.08	0.0				
T_1 x F.C.		83.13	74.03	71.13	62.27	43.63	22.00	6.54				
T_2 X F.C.		83.53	81.83	80.03	70.83	44.70	22.87	6.47				
Means	Benlate	83.22	55.89	55.47	55.17	29.89	16.11	12.33	12.23	59.74	60.07	44.01
F x F.C.	Captafol	83.67	59.50	53.17	25.44	10.28	5.44	2.17	34.55	27.98	40.19	34.24
	Copper	82.67	83.28	81.44	82.06	78.39	42.22	0.85	63.55	64.67	65.02	64.42
	Ben+Capt.	82.28	55.28	51.83	37.78	4.56	2.06	0.89	11.55	41.50	47.52	33.53
	Ben+Copper	83.11	55.09	55.22	54.44	53.89	27.33	5.44	12.21	65.21	65.95	47.79
Means (F.C.		82.99	61.81	59.43	50.98	35.40	18.63	4.34				
Means (1)									26.82	51.82	55.75	

	5% level	1% level
L S D (Strains)	2.22	2.91
L S D (Fungicides)	2.86	3.76
L S D (Fungicide conc.)	3.39	4.45
L S D (1 X F)	4.96	6.52
L S D (1 x F.C.)	5.87	7.71
L S D (F x F.C.)	7.57	9.95

concentrations were increased. This experiment shows that captafol alone and benomyl plus captafol were most effective in suppressing radial mycelial growth of strains T_1 and T_2. However, radial mycelial growth of strains T_1 and T_2 was more inhibited on MEA amended with captafol alone than when on MEA amended with benomyl plus captafol. These differences were significant at $P = 0.01$. Occasionally sectoring and zonation of cultures occurred.

4.10.3. Sporulation:

Fig 12 and Table 22 show conidial production of strains S, T_1 and T_2 on MEA amended with benomyl, captafol and Copper Nordox and their combinations. The different fungicides and interaction between fungicides and fungicide concentrations did not appear to have any significant effect on conidial production of strains T_1 and T_2. Differences among strains, fungicide concentrations and interactions between strains and fungicides were highly significant at $P = 0.001$. The interaction between strains and fungicide concentrations was significantly different at $P = 0.01$. Conidial production of strain S was greatly affected by increasing concentrations of benomyl, benomyl plus captafol and benomyl plus Copper Nordox. Captafol and Copper Nordox alone did not affect conidial production at low concentrations and at 10 and 100 µg/ml captafol, conidial production was enhanced. Although radial mycelial growth of this strain (S) was greatly reduced at higher concentrations of 500 and 1000 µg/ml captafol, there was a tendency of stimulated growth on the inoculum discs giving bunched appearance. Possibly this type of growth contributed in increased conidial production. This bunched appearance of mycelia on MEA amended with captafol was also noted for strain T_1 and T_2. Conidial production of strain S was greatly enhanced on MEA amended with captafol and Copper Nordox than when on MEA amended with benomyl, benomyl plus captafol, benomyl plus Copper Nordox. These differences were significant at $P = 0.01$. Conidial production of strains T_1 and T_2 in the presence of fungicides was enhanced

Fig. 12 : EFFECT OF BENOMYL AND OTHER FUNGICIDES ON SPORULATION OF SENSITIVE (S) AND TOLERANT (T1 & T2) STRAINS OF C. COFFEANUM

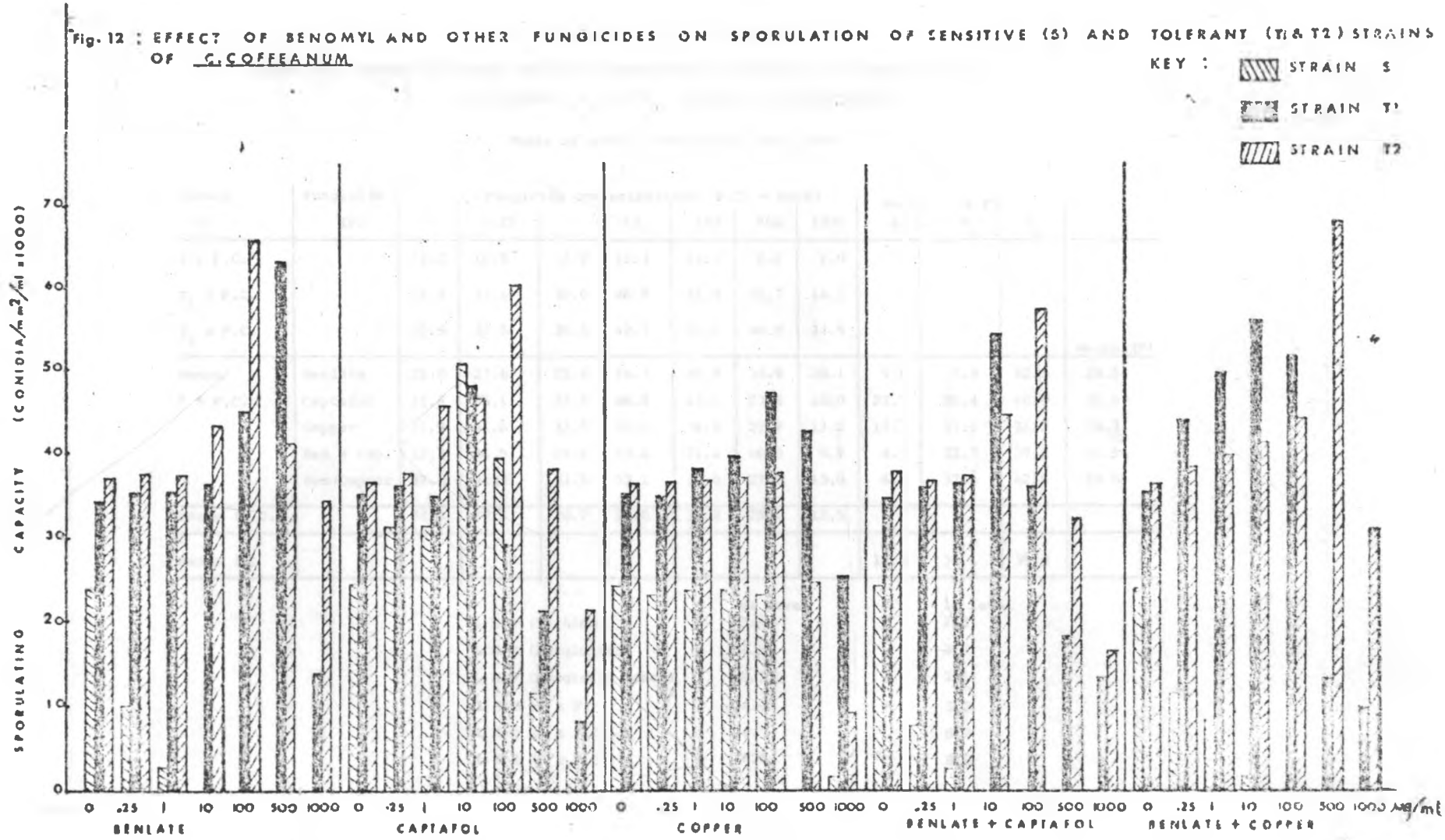


Table 22: Effect of benomyl and other fungicides on sporulation of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

Table of means (conidia/mm^2/ml x 1000)

Strain (1)	Fungicide (F)	Fungicide concentrations (F.C) - µg/ml 0	0.25	1	10	100	500	1000	Means (1 x F) S	T_1	T_2	Means (F)
S x F.C.		24.2	16.8	13.8	15.3	12.5	2.6	1.0				
T_1 x F.C.		34.9	37.4	39.0	46.9	41.9	31.7	14.1				
T_2 x F.C.		36.9	37.5	39.4	42.7	53.0	40.9	22.5				
Means	Benlate	32.0	27.8	25.4	26.7	36.9	34.9	16.1	5.4	37.8	42.5	28.5
F x F.C.	Captafol	31.8	35.1	37.4	48.5	43.1	23.6	10.9	27.5	30.4	40.9	32.9
	Copper	31.9	31.6	32.9	33.5	36.0	22.9	12.2	17.3	37.6	31.2	28.7
	Ben + Cap.	32.2	26.9	25.5	33.0	31.1	16.8	9.9	4.9	32.7	37.5	25.1
	Ben+Copper	32.0	31.3	32.5	33.1	32.0	27.0	13.6	6.5	37.1	42.8	28.8
Means (F.C.)		32.0	30.5	30.7	35.0	35.8	25.0	12.5				
Means (1)									12.3	35.1	39.0	

	5% level	1% level
L S D (Strains)	1.8	2.3
L S D (Fungicides)	2.3	3.0
L S D (Fungicide Concs)	2.7	3.6
L S D (1 x F)	4.0	5.2
L S D (1 x F.C.)	4.7	6.2
L S D (F x F.C.)	6.1	8.0

at between 0.25 and 100 μg/ml and showed a gradual decline with increasing concentrations of some fungicides and fungicide combinations. Strain T_2 maintained higher conidial production on MEA amended with 500 and 1000 μg/ml benomyl and benomyl plus Copper Nordox. Strain T_1 produced high number of conidia per ml at 500 μg/ml benomyl and Copper Nordox alone and then declined at 1000 μg/ml. Conidial production of strain T_1 was slightly less on MEA amended with captafol than when on MEA amended with benomyl plus captafol. However, these differences were not significant at $P = 0.05$. Conidial production of strain T_2 was more on MEA amended with captafol than when on MEA amended with benomyl plus captafol. These differences were not significant at $P = 0.05$. Conidial production of strain T_2 was effectively reduced on MEA amended with Copper Nordox (Table 22).

5. DISCUSSION :

The appearance of tolerance in a pathogen population may, when it is not recognised at an early stage, result in failure of disease control and consequently serious crop losses. Sometimes the use of an originally effective fungicide has to be restricted or even abandoned. Such a situation may put growers in a difficult position, if no adequate substitutes are available, and it may further reduce the financial returns of the company that developed the fungicide invariably at high cost. Benomyl proved to be very effective against coffee berry disease (CBD) and thus was widely used on coffee (Baker 1973; Okioga and Mulinge, 1974). However, Cook (1975 a) detected tolerance by the CBD pathogen to carbendazim and cypendazol in vitro and where they had been used continuously and exclusively, CBD control failed (Okioga, 1976). Because of the widespread occurrence of the tolerant strains and their large differences in sensitivity to benomyl it became necessary to carry out more investigations on the pathogen. It should be noted that the word tolerance used in this study is applied in the same context as that referred to by FAO panel of Experts of pest resistance to pesticides (1979). The tolerance in C. coffeanum strains isolated in this study seems to be of permanent nature. So far in Kenya, the perfect stage of C. coffeanum has not been identified. Thus no genetic study involving identification of genes conferring benomyl tolerance and sensitivity was undertaken.

5.1. Pathogenicity of sensitive and tolerant strains:

The benomyl tolerant strains tested were pathogenic. Hypocotyl, detached berry and attached berry infection tests indicated that the strains T_1 and T_2 were as pathogenic as the sensitive strain (S). Okioga (1976) and Javed (1980) observed that the tolerant C. coffeanum varianis were no less pathogenic than the common sensitive strain on detached coffee berries. Similar observations have been reported in other fungi (Bollen and van Zaayen, 1975; Shabi and Katan, 1979; Shalta and Sinclair, 1963;

Sozzi and Gessler, 1980; Talboys and Davies, 1976). Nevertheless, in some cases, tolerant strains are less pathogenic (Domsch, 1964; Wuest et al., 1974). Jones and Ehret (1976) observed that two benomyl tolerant isolates were similar to two sensitive isolates in virulence while a third benomyl tolerant isolate was less virulent. After 4 hr, some conidia of strain S germinated and formed appressoria. Some conidia of strains T_1 and T_2 germinated and formed appressoria at 8 and 24 hr respectively. The appressorial formation was observed at epidermal junctures and on epidermal cuticle and not on the stomata in all the three strains. Bock (1956), working with other CBD fungus isolates, reported that appressoria formed when conidia were in contact with the berry surface. Similar observations were made by Hasselbring (1906) on Gloeosporium fructigenum, Dey (1919) on Colletotrichum lindermuthianum and Dey (1933) on Colletotrichum gloeosporioides. Pathogenicity of the three strains was affected by various temperatures, conidial concentrations and benomyl concentrations. Seven days after inoculation, strain S and T_1 initiated berry infection, with active sporulation, at 15, 20 and 25°C but strain T_2 initiated berry infection, with active sporulation, at 20 and 25°C. However, at the 25 th day after inoculation all the three strains achieved high percent berry infection (above 60%), with active sporulation, at 15, 20 and 25°C. Thus, the effect of temperature on pathogenicity could not be used as a criterion to differentiate benomyl tolerant (T_1 and T_2) from sensitive (S) strains. Seven days after inoculation, strains S, T_1 and T_2 initiated berry infection, with active sporulation, with 5×10^3, 5×10^5 and 2×10^5 conidia/ml respectively. Twenty five days after inoculation, the three strains had initiated infection, with active sporulation, on a few berries with the lowest concentration of 10^3 conidia/ml. There was an increase, at 20°C ± 0.5, in percent berry infection with increasing concentration and there were indications that the optimum infection density was above 10^5 conidia/ml for the three strains. The various conidial concentrations on detached berry infection could not distinguish the benomyl tolerant from sensitive strains. Bock (1956), working with other CBD fungus isolates, found that

the optimum infection density was above 10^5 conidia/ml under laboratory conditions. However, Griffiths and Furtado (1972) were able to use very small quantities of conidia including one conidium per berry in a berry infection technique for assessing the C. coffeanum. Application of benomyl delayed the onset of infection on berries inoculated with strain S. The observations were in agreement with findings on Verticillium dahliae (Talboys et al., 1975; Talboys and Davies, 1976) where benomyl treated strawberry plants had delayed infection when inoculated with benomyl sensitive isolate. In this study, berries inoculated with strains T_1 and T_2 and treated with benomyl began to show symptoms earlier. There was also a consistent but non significant increase in berry infection in berries treated with low concentrations of benomyl. A comparable response was noted by Bollen and van Zaayen (1975) after benomyl treatment of mushrooms infected with a benomyl tolerant form of Verticillium malthousei. Similar observations were made by Talboys and Davies (1976) on benomyl treated strawberry plants inoculated with a form of V. dahliae. Javed (1980) reported that benomyl tolerant and sensitive isolates of C. coffeanum were able to infect the punctured detached green berries which had been treated with low concentrations of benomyl. Tolerance could be detected by the increased ability of the fungus to perform its normal functions of sporulation, spore germination, vegetative growth and infection in the presence of benomyl. Studies with other fungi have shown that stable benzimidazole tolerant strains arose initially by mutation from the normal strains (Dekker, 1972). This probably occurs naturally, perhaps at low frequency even in the absence of benzimidazoles. Infact a benomyl tolerant isolate of V. malthousei, a pathogen of Agaricus bisporus, was collected in 1958 before benomyl was introduced (Wuest et al., 1974). However, the actual presence of benomyl appeared to promote the production in vitro of stable tolerant variants of V. dahliae (Talboys and Davies, 1976). In the case of C. coffeanum, benomyl tolerant strains appeared to be as aggressive as the normal sensitive strain and there seemed no reason why they should rapidly disappear from the population. The exclusive use

of benomyl fungicide for the control of CBD in coffee bushes enhanced the establishment of the tolerant strains through selection pressure. The detached green coffee berry infection tests, in the presence of increasing concentrations of benomyl, differentiated benomyl tolerant from sensitive strains.

5.2. Conidial germination and germ tube characteristics, mycelial growth and sporulation of benomyl sensitive and tolerant strains:

It was observed that the rate of germination and total percent germination of the three strains was increased with increasing conidial concentrations. The lag period of germination for strains T_1 and T_2 was prolonged at lower conidial concentrations. Conidial concentration did not affect the lag period of germination of strain S. However, generally, conidia of strains T_1 and T_2 took slightly longer time to germinate in distilled sterile water compared to conidia of strain S. Germination may be considered as a growth process in which some or all the necessary nutrients are present in the spore. One could expect two extreme types of relation to external source of nutrients - complete independence in some fungi and complete dependence on one or more nutrients in others. According to these findings, all the three strains exhibited partial dependence on external nutrients in that conidia germinated after washing although total percent germination was much lower. Compared to germination in distilled sterile water, glucose dilutions increased germination rate and percent of washed conidia of strains S, T_1 and T_2 by 79.33%, 362.91% and 382.75% respectively at 2% glucose concentration. Firman and Waller (1977) reported that this effect increases as the intrinsic viability of the conidia decreases. Thus, a spore germination percentage of 57.5 was increased with the addition of 1% dextrose by 20% whereas one of 5.5 was increased by 610%. Improved nutrition also broadened the temperature range over which optimum germination occurred and raised the maximum to 35°C (Nutman and

Roberts, 1960 b). Benomyl sensitive strain S failed to germinate in 5 µg/ml benomyl whereas benomyl tolerant strains T_1 and T_2 germinated almost normally in up to 250 µg /ml benomyl solutions. Observations were made earlier by Javed (1980) that a few conidia of benomyl sensitive isolate were able to germinate in low concentrations of benomyl. Penrose and Koffman (1977) reported that benomyl sensitive isolates of Sclerotinia fructicola germinated abnormally on 1 µg/ml benomyl whereas benomyl tolerant isolates germinated almost normally on 100 µg/ml benomyl. Similar observations were made earlier by Whan (1976) on the same fungus. Conidia of strains T_1 and T_2 formed appressoria at 250 µg /ml benomyl solutions but the percent appressorial formation was significantly reduced compared to that in distilled sterile water controls. Percent appressorial formation of conidia of strains T_1 and T_2 varied with increasing benomyl concentrations. Conidia of strain S formed appressoria only up to 2.5 µg/ml benomyl. Conidial germination and appressorial formation of strains S, T_1 and T_2 was affected by each temperature regime differently. Germination of conidia of all strains occurred at all temperature regimes, 5 to 30°C, although there were variations in lag period, germination rate, total percent germination, total percent appressorial formation and type of germination. The effect of temperature on conidial germination and appressorial formation could not be used as a criterion to distinguish benomyl tolerant from sensitive strains. The tolerant strains, at a given time and particular temperature, had lower percent appressoria compared to strain S. However, the optimum range of temperature for appressorial formation for all strains was 15 to 25°C. At 30°C, germinated conidia of the three strains had long germ tubes and conidial germination to the stage of appressorial formation took much longer. Nutman and Roberts (1960 b) reported that the germination of C. coffeanum conidia in water was at about 22°C but in the presence of nutrients resulted in a broader optimum up to 27°C. They observed that maximum temperature for germination was about 30°C but up to 35°C with nutrients and a minimum of 15°C. Nutman and Roberts further observed that at optimum temperatures,

conidial germination and appressorial formation could occur within 4 hr. Conidia of strains T_1 and T_2 transferred from colonies growing on MEA amended with increasing benomyl concentrations did not lose viability. The conidia germinated in distilled sterile water at a lower percentage compared to conidia from non-amended MEA plates. No germination was observed for conidia of strain S harvested from MEA amended with 2.5 or more µg/ml benomyl. Javed (1980) observed that conidia of benomyl tolerant strains harvested from MEA amended with low benomyl concentrations did not lose their viability and were able to germinate in distilled water. Similar observations were made on Monilinia fructicola (Jones and Ehret, 1976) and V. maithousei (Wuest et al., 1974). Benomyl may be systemic within the fungus as has been demonstrated in green plants (Peterson and Edgington, 1971). If benomyl is systemic this may affect the reproductive ability and physiology of the fungus by impairing conidial formation. However, one can assume that little, if any, benomyl was present in the supernatant. This could have been possible because of the low water solubility of benomyl, especially with increasing concentrations, which lead to sedimentation in MEA culture plates. This coupled with the amount of water added to prepare concentrations of conidia harvested from benomyl amended MEA lends credence to the finding that increasing concentrations of benomyl in MEA cultures affected total percent germination of conidia of the three strains.

The three strains of C. coffeanum exhibited various colorations and a marked mycelial growth response in culture to every formulated medium. The color change on various media was either very distinct or not so distinct. Variations in mycelial coloration of CBD pathogen had been reported earlier by McDonald (1926) on a limited number of media. If the quantitative amount of mycelial growth and conidial production supported by a given medium is assumed to reflect its nutritional value to the fungus and to the survival of the fungus in nature, then certain nutrients are of much greater nutritional value. For example organic nitrogen and simple carbohydrates stimulated growth of the three strains

by increasing radial mycelial growth and conidial production. The highest conidial production /mm^2 of strains T_1 and T_2 was in medium 12 containing sucrose as the source of carbon and peptone as a source of nitrogen. Conidial production of strain S was greatly enhanced in medium 11 containing glucose as carbon source and peptone as nitrogen source although there was substantial number of conidia produced / mm^2 in medium 12. All the three strains had good radial mycelial growth in medium containing coffee berry extracts although conidial production was slightly suppressed. The various media used in this study could not distinguish benomyl tolerant from sensitive strains. There were no significant differences in radial mycelial growth among the strains. It was also noted that conidial production /mm^2 of strain S seemed to be significantly lower than that of strains T_1 and T_2. The essentiality of heavy metals, such as zinc, iron and copper, in the nutrition of the three strains was demonstrated in these experiments. Significant differences were found in radial mycelial growth and conidial production /mm^2 in all strains growing on media amended with heavy metals, zinc, copper and iron, compared to non-amended media. However, the interaction between heavy metals and strain was not significant. Thus, the incorporation of heavy metals in media could not distinguish benomyl tolerant from sensitive strains. The importance of heavy metals in the nutrition of fungi had been recognised since the very first studies dealing with the cultivation of filamentous fungi on synthetic media. Raulin's (1869) classical researches (cited by Foster, 1939) on the growth of Ascophora nigrans (Aspergillus niger) clearly demonstrated that in addition to the usual mineral nutrients such as potassium, magnesium, phosphorus etc certain other elements, notably zinc and iron, must be furnished in the medium in order to obtain the maximum dry weight of the fungus. The amounts of heavy metal ions which are effective in obtaining maximum growth of fungi are so minute compared to the more familiar mineral constituents of synthetic media that the term "trace" elements is often applied to them. The relation of nutrition and parasitism of CBD pathogen is highly speculative. It

is possible that nutritional status of coffee berries may exert some influence on the susceptibility of the berries to the CBD. As no studies were undertaken of the nutritional status of coffee trees, the data reported here are inconclusive as to the role played by various nutrients status of the host in determining the inception of the CBD. The ability of C. coffeanum strains to produce color variants by sectoring in culture was also observed, in other isolates, by Okioga (1976). Strains T_1 and T_2 grew normally on MEA with as high as 1000 µg/ml benomyl although the colony diameters were reduced gradually with increasing concentrations whereas strain S showed little mycelial growth at low benomyl concentrations. Javed (1980) reported that tolerant isolates of C. coffeanum continued to grow on MEA with 1000 µg/ml Benlate. Similar observations were reported in other fungal pathogens, Sclerotinia fructicola (Penrose and Koffman, 1977; Whan, 1976), Penicillium spp. (Bollen, 1971), Verticillium fungicola (Bollen and van Zaayen, 1975), Botrytis cinerea (Geeson, 1976; Bollen and Scholten, 1971), Monilinia fructicola (Jones and Ehret, 1976), Fusarium Oxysporum f.sp. lycopersici (Sozzi and Gessler, 1980), Verticillium malthousei (Wuest et al., 1974). The strains showed marked variations in sporulation. The presence of up to 250 µg/ml benomyl in MEA stimulated conidial production of strain T_2. This phenomenon of variations in sporulation has been reported before (Jones and Ehret, 1976; Wuest et al., 1974). It was also observed that mycelial growth of strains T_1 and T_2 was less sensitive to benomyl than conidial germination. It was not possible to grow colonies on MEA amended with 500 and 1000 µg/ml benomyl using conidia for inoculation. None of the benomyl tolerant strains, T_1 and T_2, showed partial or intermediate tolerance to benomyl. Incorporation of various benomyl concentrations in MEA differentiated benomyl tolerant from sensitive strains. There were significant differences in radial mycelial growth and conidial production/mm^2 between benomyl tolerant and sensitive strains. It was also noted that conidial production/mm^2 of strain S on non-amended MEA seemed to be lower than that of strains T_1 and T_2. Conidial measurements and microscopic observations

showed no evidence of reduction in size or change in morphology of conidia when the three strains were grown on benomyl amended MEA. Similar observations were made by Littrel (1974) with Cercospora arachidicola. This is not in agreement with observations by Griffee (1973) with Colletotrichum musae and Javed (1980) with benomyl tolerant isolates of C. coffeanum in which conidial size was reduced by culturing on a benomyl amended medium. Strains T_1 and T_2 were also tolerant to two other benzimidazole fungicides (Derosal and Bavistin). Their tolerance to these two fungicides, in terms of mycelial growth and sporulation, was more striking with increasing concentrations than to that of benomyl. Cross tolerance of tolerant isolates of C. coffeanum has been reported earlier (Cook and Pereira, 1977; Okioga 1976). The occurrence of cross tolerance to other benzimidazole fungicides exhibited by the two strains was similar to observations made on other benomyl tolerant fungi (Bollen, 1971; Bollen and Scholten, 1971; Bollen and van Zaayen, 1975; Clark et al., 1974; Giannopolitis, 1978; Jones and Ehret, 1976; Littrell, 1974; Muirhead, 1974; Penrose and Koffman, 1977; Smiley and Howard, 1976; Warren et al., 1974; Wicks, 1974). Tolerance and cross tolerance has been reported in Rhizoctonia bataticola to non-benzimidazole fungicides (Anikumar and Sastry, 1979).

5.3. The effect of subculturing benomyl tolerant strains:

Strains T_1 and T_2 were subcultured for six months without loss of tolerance. Subculturing affected conidial production of strains S, T_1 and T_2 although strains T_1 and T_2 were able to grow and produce conidia at 1000 µg/ml benomyl. Conidial production was low for all the repeatedly subcultured "isolates" compared to "isolates" subcultured only once on fungicide free MEA. The loss in the ability to produce conidia could be attributed to subculturing rather than the initial treatment with benomyl. Conidia of strains T_1 and T_2 germinated and formed appressoria in up to 250 µg/ml benomyl concentration. Stability of benomyl tolerance has been reported on other fungal pathogens. Bollen (1971)

reported that the strains of Penicillium spp. retained their resistance at the same level for at least three months after repeated subculturing on fungicide free agar. Resistance of isolates of Botrytis cinerea was retained for at least 20 weeks after repeated subculturing on fungicide free agar (Bollen and Scholten, 1971). Tolerance of two benomyl tolerant isolates of Monilinia fructicola was unchanged after 13 passages through benomyl free substrates including four passages through peach fruit (Jones and Ehret, 1976). Carbendazim resistant isolates of Venturia pirina retained tolerance after subculturing for over one year (Shabi and Katan, 1979). Subculturing did not seem to affect pathogenicity of the three strains on detached berries of LS 28. However, infectivity on detached berries was slightly reduced. This could be due to the much longer germ tubes formed before appressorial formation in germinating conidia of repeatedly subcultured "isolates".

5.4. Development of tolerance:

Germ tube and hyphal fusions were observed in all the three strains. According to Alexopoulos (1962) there are several ways in which a heterokaryotic mycelium may be formed. The most common perhaps is by anastomosis of somatic hyphae of different genetic constitutions. Genetic studies of fungicide resistance have been carried out by various research workers (Georgopoulos, 1977). For these studies fungi with a perfect stage were used, which allowed genetic analysis for the recognition of genes conferring resistance. Studies were carried out with non-pathogens, such as Emiricella nidulans (imperf. Aspergillus nidulans), Neurospora crassa and Saccharomyces cerevisiae, and with pathogens, such as Venturia inaequalis, Ustilago maydis, U. hordei and Nectria haematococca. Genetic aspects of fungicide resistance in imperfecti fungi can be studied when the fungi have a parasexual cycle. The resulting heterokaryotic mycelium will also be having genes for tolerance and possibly will confer tolerance to benomyl in new mycelia formed from the germinating conidia. Thus tolerance to benomyl will

persist in the population of the pathogen. Such studies would have direct practical importance, since the CBD fungus tolerant strains were found to be cross-resistant to other benzimidazole fungicides with similar mechanism of action. Mixed inoculation tests with different strains in vivo gave no indication of a decline of tolerant strains. This study showed that in the absence of selection pressure by benomyl, the tolerant strains were not weaker and tended to take over. This could be partly explained by the fact that anastomosis of germ tubes and somatic hyphae of the strains of C. coffeanum and high conidial production by tolerant strains, T_1 and T_2, could have made tolerant strains better competitors. Similar observations have been made on other fungal pathogens for example Fusarium oxysporum f.sp. lycopersici (Sozzi and Gessler, 1980), Cladosporium cucumerinum (Dekker, 1969), Monilinia fructicola (Jones and Ehret, 1976), Venturia pirina (Shabi and Katan, 1979), Botrytis cinerea (Miller and Jeves, 1979). This is not in agreement with Domsch (1964) who reported that tolerant strains were poor competitors. Samples taken from attached berries in the field after 5½ months of inoculation showed little decline in the incidence of tolerant strains in the absence of benomyl. Tolerance seemed to be stable in vivo in the absence of benomyl. This suggested a permanent change in the genetic material rather than conditional adaptation. Dovacs et al. (1976) reported that four years after cessation of benomyl applications the frequency with which tolerant strains of Cercospora beticola Sacc. were isolated from sugar beet had not changed. Similar observations were made by Miller and Jeves (1979) on Botrytis cinerea in glasshouse tomato plants. It seems therefore, that tolerant strains of C. coffeanum are able to compete successfully with sensitive strains. The observations that both tolerant and sensitive conidia could be present within the same lesion is important. A false impression of the prevalence of benomyl tolerance within a crop could be obtained if lesions were only screened for the presence or absence of tolerance. The lesions with both tolerant and sensitive strains of the fungus may have resulted from the movement of conidia within the coffee bush, from two or more distinct strains growing in close association or from a single heterokaryotic strain.

Strains heterokaryotic for benomyl tolerance have been demonstrated (Pourtois et al., 1976). If present, these strains could be important in maintaining a pool of tolerant nuclei in the population in the absence of selection pressure.

5.5. In vitro control of benomyl tolerant strains:

Although the three strains exhibited significant variations in mycelial growth, sporulation and conidial germination, strains T_1 and T_2 were as vigorous as strain S and tolerance was stable in vitro and in vivo. Strains T_1 and T_2 have shown sensitivity to standard fungicides, captafol 80% w.p. and copper 50% formulation. The sensitivity was only observed at concentrations of 500 and 1000 µg/ml a.i. captafol and copper 50% formulation. This study indicated that there was no synergism, under laboratory conditions, between benomyl and captafol or Copper Nordox. In the presence of benomyl, alone or in combination, mycelial growth, sporulation and conidial germination of tolerant strains were not adversely affected. Sporulation, mycelial growth and conidial germination of strain S were strongly inhibited by benomyl, benomyl plus captafol and benomyl plus Copper Nordox concentrations of 1 µg/ml and higher. Neither captafol nor Copper Nordox was as active as benomyl at low concentrations against strain S. Similar observations were made by Jones and Ehret (1976) on sensitive strain of Monilinia fructicola. Presumably tolerant strains would decline if they were less aggressive or if they had increased sensitivity to standard contact fungicides as shown by benomyl tolerant strains of Verticillium malthousei (Lambert and Wuest, 1975) and Cercospora beticola (Ruppel, 1975). Growers who decide to use the combination treatments should be advised to monitor CBD carefully in their coffee trees and to switch immediately to an alternative material if CBD begins to develop to high levels. Conflicting observations in the control of tolerant strains have been reported. Sutton (1978) reported that strains of Venturia inaequalis tolerant to benomyl were not controlled under orchard conditions with combination treatments at reduced rates of

benomyl and either Captan or Zinc ion - Maneb Complex. However, under the same conditions full rates of dodine or captafol were quite effective against tolerant strains of V. inaequalis. Chandler et al. (1978) reported that benomyl used alone was ineffective against benomyl tolerant Cladosporium carpophilum but effective against M. fructicola. They further observed that benomyl continued to be very effective against C. carpophilum where tolerance had not developed. The non-systemic standard fungicides, captafol 80% w.p. and copper 50% formulation, which acted to prevent germination and affected germ tube growth respectively might effectively control benomyl tolerant strains at high concentrations. Penrose and Koffman (1977) reported that non-systemic fungicides, Captan and glycophene, which act to prevent germination were effective in controlling benomyl tolerant strains of Sclerotinia fructicola.

CONCLUSIONS

Tolerance in C. coffeanum strains isolated in this study seemed to be of permanent nature. The benomyl tolerant strains, T_1 and T_2, tested were as pathogenic as the sensitive strain, S. The appressorial formation was observed at epidermal junctures and on epidermal cuticle and not on the stomata in all the three strains. At the 25th day after inoculation all the three strains achieved high percent berry infection (above 60%), with active sporulation, at 15, 20 and 25°C. The effect of temperature on pathogenicity could not be used as a criterion to differentiate benomyl tolerant from sensitive strains. There was an increase, at 20°C ± 0.5, in percent berry infection with increasing concentration and there were indications that the optimum infection density was above 10^5 conidia/ml for the three strains. The various conidial concentrations on detached berry infection could not distinguish the benomyl tolerant from sensitive strains under laboratory conditions. Application of benomyl delayed the onset of infection on berries inoculated with strain S. Berries inoculated with strain T_1 and T_2 and treated with benomyl began to show symptoms earlier. The detached green coffee berry infection tests, in the presence of increasing concentrations of benomyl, differentiated benomyl tolerant from sensitive strains. Benomyl tolerant strains appeared to be as agrressive as the sensitive strain and there seemed no reason why they should rapidly disappear from the population. Conidia of strains T_1 and T_2 took slightly longer time to germinate in distilled sterile water compared to conidia of strain S. The rate of germination and total percent germination of the three strains was increased with increasing conidial concentrations. All the strains exhibited partial dependence on external glucose in that conidia germinated after washing although total percent germination was much lower. Compared to germination in distilled sterile water, glucose dilutions increased germination rate and percent of washed conidia of strains S, T_1 and T_2 by 79.33%, 362.91% and 382.75% respectively at 2% glucose concentration. Strain S failed to germinate and form appressoria in 5 µg/ml benomyl whereas strains T_1 and T_2 germinated and

formed appressoria normally in up to 250 µg/ml benomyl. Conidial germination of all strains occurred at 5 to 30°C, although there were variations in lag period, germination rate, total percent germination, total percent appressorial formation and type of germination. The effect of temperature on conidial germination and appressorial formation could not be used as a criterion to distinguish benomyl tolerant from sensitive strains. Conidia of strains T_1 and T_2 transferred from colonies growing on MEA amended with increasing benomyl concentrations did not lose viability. No germination was observed for conidia of strain S harvested from MEA amended with 2.5 or more µg/ml benomyl. The three strains exhibited various colourations and a marked mycelial growth response in culture to every formulated medium. Organic nitrogen, peptone, and simple carbohydrates, glucose and sucrose, stimulated growth of the three strains by increasing radial mycelial growth and conidial production. All the strains had good radial mycelial growth in medium containing berry extracts although conidial production was slightly suppressed. The various formulated media, with or without zinc, copper and iron, could not distinguish benomyl tolerant from sensitive strains. Strains T_1 and T_2 grew normally on MEA with as high as 1000 µg/ml benomyl although the colony diameters were reduced gradually with increasing concentrations whereas strain S showed little mycelial growth at low benomyl concentrations. It was also noted that conidial production/mm^2 of strain S on non-amended MEA seemed to be lower than that of strains T_1 and T_2. Conidial measurements and microscopic observations showed no evidence of reduction in size or change in morphology of conidia when the three strains were grown on benomyl amended MEA. Incorporation of various benomyl concentrations in MEA differentiated benomyl tolerant from sensitive strains. Benomyl tolerant strains, T_1 and T_2, showed cross tolerance to two other benzimidazole fungicides, Derosal and Bavistin. Strains T_1 and T_2 were subcultured for six months without loss of tolerance. Subculturing did not seem to affect pathogenicity of the three strains on detached berries of SL 28. Germ tube and hyphal fusions were observed in all the three strains. Mixed inoculation tests with different strains

in vivo gave no indication of a decline of tolerant strains and in the absence of selection pressure by benomyl the tolerant strains were not weaker but tended to take over. Samples taken from inoculated attached berries in the field after 5½ months of inoculation showed little decline in the incidence of tolerant strains in the absence of benomyl. This suggested a permanent change in the genetic material rather than conditional adaptation. Strain T_1 and T_2 showed sensitivity to standard fungicides viz. captafol 80% w.p. and Copper Nordox 50% w.p. at 500 to 1000 µg/ml a.i. under laboratory conditions. Neither captafol 80% w.p. no Copper Nordox 50% w.p. was as active as benomyl at low concentrations against strain S. The non-systemic fungicides, captafol 80% w.p. and Copper Nordox 50% w.p. which acted to prevent germination and affected germ tube growth respectively might effectively control benomyl tolerant strains at high concentrations. Growers who decide to use the combination treatments should be advised to monitor CBD carefully in their coffee trees and to switch immediately to an alternative fungicide if CBD begins to develop to high levels.

LITERATURE CITED

1. AGNIHOTHRUDU, V. (1972) Fungi associated with the new malady of coffee in South India. Proceedings of the Indian academy of Sciences, B 75 : 55 - 64.

2. ALANDIA, S,& BELL, F.H. (1957) Diseases of warm climate crops in Bolivia. F A O Plant Protection Bulletin 5 : 172 - 173.

3. ANDES, T.O.& KEITT, G.W. (1950) Variability of Glomerella cingulata (Stonem.) S & v.S. Phytopathology 40 : 915 - 925.

4. ALEXOPOULOS, C.J. (1962) Introductory Mycology pp. 394 - 396, 2nd edition, John Wiley & Sons, Inc., New York, London; TOPPAN Co. LTD., TOKYO, Japan.

5. ANILKUMAR, T.B.& SASTRY, M.N. (1979) Development of tolerance to fungicides in Rhizoctonia bataticola. Phytopath. Z. 94 : 126 - 131.

6. ANON. (1934) Report of Committee on coffee berry disease. Bulletin of the Development of Agriculture, Kenya No. 3.

7. ANON. (1943) American phytopathological society, Committee on standardization of fungicidal tests. Standard Laboratory Bordeaux mixture. Phytopathology 33 : 633 - 634.

8. ANON, (1947) American phytopathological Society, Committee on standardization of fungicidal tests. The slide germination method of evaluating protectant fungicides. Phytopathology 37: 354 - 356.

9. ANON. (1958) Colonial Research 1957 - 8. 13th Annual Report of the Committee for colonial Agriculture, Animal Health and Forestry Research: 92.

10. ANON. (1965) Annual Report of the Coffee Research Foundation, Kenya 1964.

11. ANON. (1975) Better coffee farming. Control of coffee berry disease (CBD) and leaf rust. Kenya Coffee 40: 211-214.

12. ANON. (1976) An Atlas of Coffee Pests and Diseases pp 102 - 102B 5th edition, Coffee Board of Kenya.

13. ANON. (1978/79) Annual Report, Coffee Board of Kenya.

14. ANON (1979/80) Annual Report, Coffee Board of Kenya.

15. BAKER, C.J. (1973) 1972 Trials with new and recommended fungicides. Kenya Coffee 38 : 185 - 191.

16. BERGER, R.D. (1973) Disease Progress of Cercospora apii resistant to benomyl. Plant Disease Reporter 57 : 837 - 840.

17. BOCK, K.R. (1956) Investigations of coffee berry disease - Laboratory studies. East African Agricultural Journal 29 : 97 - 103.

18. BOCK, K.R. (1963) The control of coffee berry disease in Kenya. Empire Journal of Experimental Agriculture 31 : 97 - 107.

19. BOCK, K.R. (1970) Plant Pathology in East Africa. Review of Plant Pathology 49 : 1 -6.

20. BOCK, K.R. & RAYNER, R.W. (1956) Control of coffee berry disease in Kenya. Nature, U K. 178 : 217 - 218.

21. BOCK, K.R. ; ROBINSON, J.B.D. & CHAMBERLAIN, G.T. (1958) Zinc deficiency induced by mercury in Coffea arabica. Nature, U K 182: 1607-1608.

22. BOISSON, C. (1960) L'anthracnose du Cafeier. Revue de Mycologie 25 : 263 - 292.

23. BOLLEN, G.J. (1971) Resistance to benomyl and some chemically related compounds in strains of Penicillium Spp. Neth. J. Plant Pathology 77 : 187 - 193.

24. BOLLEN, G.J.& SCHOLTEN, G. (1971) Acquired resistance to benomyl and some other systemic fungicides in a strain of Botrytis cinerea in cyclamen. Neth. J. Plant Pathology 77 : 83 - 90.

25. BOLLEN, G.J.& van ZAAYEN, A. (1975) Resistance to benzimidazole fungicides in pathogenic strains of Verticillium fungicola. Neth. J. Plant Pathology 81 : 157 - 167.

26. BUTLER, E.J. (1918) Fungi and diseases in plants. Calcutta, Thacker, Spink & Co. 547 pp.

27. BUTT, D.J. & BUTTERS, B. (1966) The control of coffee berry disease in Uganda. Report of the First Specialist Meeting of Coffee Research in East Africa. Nairobi, East African Common Services Organisation.

28. CHANDLER, W.A., DANIELL, J.W. & LITTRELL, R.H. (1978) Control of peach diseases in an orchard having benomyl tolerant Cladosporium carpophilum. Plant Disease Reporter 62 : 983 - 986.

29. CLARK, E.M., BACKMAN, P.A. & RODRIGUEZ - KABANA, R. (1974) Cercospora and Cercosporidium tolerance to benomyl and related fungicides in Alabama peanut fields. Phytopathology 64 : 1476 - 1477.

30. CLEMENTS, F.E. & SHEAR, C.L. (1957) The genera of fungi. Hafner, New York. 496 pp.

31. CLEMONS, G.P. & SISLER, H.D. (1969) Formation of a fungitoxic derivative from Benlate. Phytopathology 59 : 705 - 706.

32. COOK, R.T.A. (1971) Screening for CBD resistance. I. CBD spore production. Unpublished.

33. COOK, R.T.A. (1973) Detecting disease resistance in coffee plants. Kenya Coffee 38 (No.450) : 275 - 277.

34. COOK, R.T.A. (1975 a) The future of benzimidazole fungicides for CBD control in Kenya. Kenya Coffee 40 : 139 - 144.

35. COOK, R.T.A. (1975 b) The effect of weather conditions on infection by coffee berry disease. Kenya Coffee 40 : 190 - 197.

36. COOK, R.T.A. (1976) Methods of screening Coffea arabica plants for resistance to Colletotrichum coffeanum, the causal agent of coffee berry disease in Kenya. In press.

37. COOK, R.T.A. & PEREIRA, J.L. (1977) Strains of Colletotrichum coffeanum, the causal agent of coffee berry disease, tolerant to benzimidazole compounds in Kenya. Kenya Coffee 42 (No.491) : 63 - 76.

38. COOK, R.T.A. & van der VOSSEN, H.A.M. (1974) Screening coffee plants for CBD resistance. Annual Report, Coffee Research Foundation, Kenya 1973/74 : 43, 44 & 64.

39. CRAMER, H.H. (1967) Plant Protection and World Crop Production. Leverkusen, Farbenfabriken Bayer Ag. 524 pp.

40. Da PONTE, A.M. (1966) Spraying arabica coffee with calcium superphosphate for control of coffee berry disease usually attributed to Colletotrichum coffeanum Noack. Kenya Coffee 31 : 21 - 22.

41. DEKKER, J. (1969) Acquired resistance to fungicides. World Review of Pest Control 8 (2) : 79-85.

42. DEKKER, J. (1972) Resistance. In systemic fungicides, Editor, R.W. Marsh . Ist ed. William Clowes & Sons Ltd., London, Longmans. 321 pp.

43. DEKKER, J.& GIELINK, A.J. (1979) Acquired resistance to pimaricin in Cladosporium cucumerinum and Fusarium oxysporium f. sp. narcissi associated with decreased virulence. Neth. J. Plant Pathology 85 (2) : 67 - 73.

44. DEY, P.K. (1919) Studies in the physiology of parasitism. V. Infection by Colletotrichum lindemuthianum. Ann. Botany xxxiii : 305.

45. DEY, P.K. (1933) Studies in the physiology of the appressorium of Colletotrichum gloeosporioides. Ann. Botany 47 : 305 - 312.

46. DOMSCH, K.H. (1964) Soil fungicides. Annual Review of Phytopathology 2 : 293.

47. DOVAGS, C., SKYLAKAKIS, G.& G. GEORGOPOULOS, S.G. (1976) The adaptability of the benomyl resistant population of Cercospora beticola in Northern Greece. Phytopathology 66 : 1452 - 1456.

48. F.A.O. panel of experts on pest resistance to pesticides (1979). Report meeting 28 August - 1 September 1978 AGP: 1979/M/2. pp.41.

49. FIRMAN, I.D.& WALLER, J.M. (1977) Coffee berry disease and other Colletotrichum diseases of coffee. Phytopathological papers, No.20 ISBN 0 85198 367 7.

50. FERNIE, L.M. & VERMEULEN, H. (1966) The screening of Lyamungu material for resistance to coffee disease. Report of the First Specialist Meeting on Coffee Research in E.Africa. Nairobi, East Africa Common Services Organisation.

51. FOSTER, J.W. (1939) The heavy metal nutrition of fungi. The Botanical review V (No. 4) : 207 - 239.

52. FROST, E.R. (1964) Seta formation in Colletotrichum species. Nature, U.K. 201 : 730 - 731.

53. GEESON, J.D. (1976) Comparative studies of methyl-benzimidazole-2-ylcarbamate - tolerant and sensitive isolates of Botrytis cinerea and other fungi. Trans. Brit. Mycol. Soc. 66 : 123 - 129.

54. GEORGOPOULOS, S.G. (1977) Development of fungal resistance to fungicides. Editors, M.R. Siegel & H.D. Sisler, Antifungal compounds, vol. 2. Marcel Dekker Inc., New York and Basel, pp.439-495.

55. GEORGOPOULOS, S.G.& DOVACS, C. (1974) A serious outbreak of strains of Cercospora beticola resistant to benzimidazole fungicides in Northern Greece. Plant Disease Reporter 57 : 321 - 324.

56. GIANNOPOLITIS, C.N. (1978) Occurrence of strains of Cercospora beticola resistant to Triphenyltin fungicides in Greece. Plant Disease Reporter 62 : 205 - 208.

57. GIBBS, J.N. (1968) Annual Report of the Coffee Research

Foundation, Kenya, 1967/68 : 44 - 46.

58. GIBBS, J.N. (1969) Inoculum sources for coffee berry disease. Annals of Applied Biology 64 : 515 - 522.

59. GIBBS, J.N. (1971) Some factors affecting the performance of spray programmes for the control of coffee berry disease. Annals of Applied Biology 67 : 343 - 356.

60. GRIFFEE, P.J. (1973) Resistance to benomyl and related fungicides in Colletotrichum musae. Trans. Brit . Mycol. Soc. 60 : 433 - 439.

61. GRIFFITHS, E.& GIBBS, J.N. (1969) Early season sprays for the control of coffee berry disease. Annals of Applied Biology 64 : 523 - 532.

62. GRIFFITHS, E. ; GIBBS, J.N.& WALLER, J.M. (1971) Control of coffee berry disease. Annals of Applied Biology 67 : 43 - 74.

63. GRIFFITHS, E.& FURTADO, I. (1972) A berry infection technique for assessment of the CBD strain of Colletotrichum coffeanum on coffee branches. Trans. Brit. Mycol. Soc. 58 : 313 - 320.

64. GRIFFITHS, E.& WALLER, J.M. (1971) Rainfall and cropping patterns in relation to coffee berry disease. Annals of Applied Biology 67 : 75 - 91.

65. HAMMERSCHLAG, R.S. & SISLER, H.D. (1972) Benomyl and methyl-2-benzimidazole carbamate (MBC): Biochemical, cytological and chemical aspects of

toxicity to Ustilago maydis and Saccharomyces cerevesiae. Pesticide Biochemistry and physiology 3 : 42 - 54.

66. HASSELBRING, H. (1906) The appressoria of the anthrachnoses. Bot. Gaz. xlii : 135.

67. HINDORF, H. (1970) Colletotrichum species isolated from Coffea arabica in Kenya. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 77 : 328 - 331.

68. HINDORF, H. (1973 a) Colletotrichum population on Coffea arabica in Kenya. I. A method for the systematic separation of fungus populations. Phytopath. Z. 77 : 97 - 116.

69. HINDORF, H. (1973 b) Colletotrichum population on Coffea arabica in Kenya. II. Qualitative and quantitative differences in the Colletotrichum population. Phytopath. Z. 77 : 216 - 234.

70. HINDORF, H. (1973 c) Colletotrichum population on Coffea arabica in Kenya. III. The distribution of Colletotrichum Spp. on different parts of the coffee bush. Phytopath. Z. 77 : 324 - 338.

71. HINDORF, H. (1974) Colletotrichum species from coffee growing areas of the Kiambu District of Kenya. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 81 : 108 - 113.

72. HINDORF, H &MUTHAPPA, B.N. (1974) A comparison of Colletotrichum coffeanum Noack from South India

and Kenya. Phytopath Z. 80 : 9 - 12.

73. HOCKING, D. (1966) Brown blight (Colletotrichum coffeanum Noack) of arabica in East Africa. Annals of Applied Biology 58 : 409 - 421.

74. HOCKING, D. (1971) Alternative hosts for two races of Colletotrichum coffeanum from coffee. Turrialba 21 : 234 - 235.

75. HOCKING, D., JOHANNS, J.C. & VERMEULEN, H. (1967) Ascospore production, discharge and infection by Glomerella cingulata causing coffee berry disease. Nature, U K . 214 : 1144 - 1145.

76. HOITINK, H.A.J. & SCHMITHENNER, A.F. (1970) Disease control in rhododendron cuttings with benomyl or thiabendazole in hormone mixtures. Plant Disease Reporter 54 : 427 - 430.

77. HUBBELING, N. (1957) New aspects of breeding for disease resistance in beans (Phaseolus vulgaris L.) Euphytica 6 : 111 - 141.

78. JAVED, Z.U.R. (1978) Spray timing. Annual Report of Coffee Research Station, Ruiru, Kenya 1976/77, 126 pp.

79. JAVED, Z.U.R. (1980) Benomyl tolerance in Colletotrichum coffeanum, the cause of coffee berry disease. Kenya coffee 45 (No 529) : 123 - 127.

80. JONES, A.L.& EHRET, G.R. (1976) Isolation and characterization of benomyl tolerant strains of Monilinia fructicola. Plant Disease Reporter 60: 765 - 769.

81. JONES, A.L.& WALKER, R.J. (1976) Tolerance of Venturia inaequalis to dodine and benzimidazole fungicides in Michigan. Plant Disease Reporter 60 : 40 - 44.

82 KAISER, W.J.& LUKEZIC, F.L. (1966) Occurrence, sporulation and pathogenicity studies with Glomerella cingulata associated with crown rot of boxed bananas. Mycologia 58 : 397 - 405.

83. KIRKPATRICK, T.W. (1935) The climate and eco-climate of coffee plantations. Amani memoirs. Crown Agents for the Colonies.

84. LAMBERT, D.P.& WUEST, P.J. (1975) Increased sensitivity to Zineb for Verticillium malthousei strains tolerant to benomyl. Phytopathology 65 : 637 - 638.

85. LAMPARD, J.F.& CARTER, G.A. (1973) Chemical investigations on resistance to coffee berry disease in Coffea arabica. An antifungal compound in coffee cuticular wax. Annals of Applied Biology 73 : 31 - 37.

86. LITTRELL, R.H. (1974) Tolerance in Cercospora arachidicola to benomyl and related fungicides. Phytopathology 64 : 1377 - 1378.

87. MASTENBROEK, C. (1960) A breeding programme for resistance to anthracnose in dry shell haricot beans, based on new

gene. Euphytica 9 : 177 - 184.

88. MAXWELL, W.A.& BROODY, G. (1971) Antifungal activity of selected benzimidazole compounds. Applied microbiology 21 : 944 - 945.

89. McDonald, J. (1926) A preliminary account of a disease of green coffee berries in Kenya. Trans. Brit. Mycol Soc. 11 : 145 - 154.

90. McDonald, J. (1932) Annual Report of the Department of Agriculture, Kenya, 1931 : 119.

91. MEREDITH, D.S. (1964) Appressoria of Gloeosporium musarum Cooke & Massee on banana fruits. Nature U.K. 201: 214 - 215.

92. MILLER, M.W.& JEVES, T.M. (1979) The persistence of benomyl tolerance in Botrytis cinerea in glasshouse tomato crops. Plant Pathology 28 : 119 - 122.

93. MUIRHEAD, I.F. (1974) Resistance to benzimidazole fungicides in blue mould of citrus in Queensland. Austral. J. Exp. Agric. Anim. Husb. 14 : 698 - 701.

94. MULINGE, S.K. (1970) Development of coffee berry disease in relation to the stage of growth of the berry. Annals of applied Biology 65: 269 - 276.

95. MULINGE, S.K. (1973) Outbreaks and new records in Ethiopia, coffee berry disease. FAO Plant Protection Bulletin 21 : 85 - 86.

96. NUTMAN, F.J. (1966) Coffee berry disease - a review. Report of the first specialist meeting of Coffee Research in East Africa. Nairobi, East African Common Services Organisation.

97. NUTMAN, F.J. (1970) Coffee berry disease. PANS 16: 277 - 286.

98. NUTMAN, F.J.& ROBERTS, F.M. (1960 a) Investigations on a disease of Coffea arabica caused by a form of Colletotrichum coffeanum Noack. I. Some factors affecting infection by the pathogen. Trans. Brit. Mycol. Soc. 43 : 489 - 505.

99. NUTMAN, F.J.& ROBERTS, F.M. (1960 b) Investigations on a disease of Coffea arabica caused by a form of Colletotrichum coffeanum Noack. II. Some factors affecting germination and infection and their relation to disease distribution. Trans. Brit. Mycol. Soc. 43 : 643 - 659.

100. NUTMAN, F.J.& ROBERTS, F.M. (1961) Investigations on a disease of Coffea arabica caused by a form of Colletotrichum coffeanum Noack. III. The relation between infection of bearing wood and disease incidence. Trans Brit. Mycol. Soc. 44 : 511 - 521.

101. NUTMAN, F.J.& ROBERTS, F.M. (1962) Stimulation of two pathogenic fungi by high dilutions of fungicides. Trans. Brit. Mycol. Soc. 45 : 449 - 456.

102. NUTMAN, F.J.& ROBERTS, F.M. (1969 a) Seasonal variations in the sporulating capacity of the fungus

causing coffee berry disease. Annals of Applied Biology 64 : 85 - 99.

103. NUTMAN, F.J.& ROBERTS, F.M. (1969 b) Climatic conditions in relation to the spread of coffee berry disease since 1962 in the East Rift districts of Kenya. East African Agricultural and Forestry Journal 35 : 118 - 127.

104. NUTMAN, F.J.& ROBERTS, F.M. (1970). A note on a laboratory technique for testing fungicides against coffee berry disease. East African Agricultural and Forestry Journal 35 : 225-228.

105 OKIOGA, D.M. (1976) Occurrence of strains of Colletotrichum coffeanum resistant to methyl benzimidazol-2-ylcarbamate (carbendazim) and chemically similar compounds. Annals of Applied Biology 84 : 21-30.

106. OKIOGA, D.M.& MULINGE, S.K. (1974) 1973 trials with new and recommended fungicides. Kenya Coffee 39 : 319 - 322.

107. PENROSE, L.J.& KOFFMAN, W. (1977) Tolerance of Sclerotinia fructicola to benzimidazole fungicides and control of the fungus. Phytopath Z. 88 : 153 - 164.

108. PETERSON, C.A.& EDGINGTON, L.V. (1971) Transport of benomyl into various plant organs. Phytopathology 61 : 92.

109. POURTOIS, A.; MARAITE, H.& MEYER, J.A. (1976) Distribution de tolerance au benomyl dans less populations de

Botrytis cinerea Pers. Anneles de phytophalogie 8 : 1 - 8.

110. RAYNER, R.W. (1941) Report of Plant Pathologist (Coffee service). Report of the Department of Agriculture, Kenya, Unpublished.

111. RAYNER, R.W. (1948) Latent infection in Coffea arabica. Nature, U K 161: 245 - 246.

112. RAYNER, R.W. (1952) Coffee berry disease. A survey of investigations carried out up to 1950. East African Agricultural Journal 17 : 130 - 158.

113. RIDDELL, R.W. (1950) Permanent stained mycological preparation obtained by slide culture. Mycologia 42 : 265 - 270.

114. ROBINSON, R.A. (1974) Terminal report of the FAO coffee Pathologist to the Government of Ethiopia , FAO, Rome. 17 pp.

115. RUPPEL, E.G. (1975) Biology of benomyl tolerant strains of Cercospora beticola from sugar beet. Phytopathology 65 : 785 - 789.

116. SACCAS, A.M.& CHARPENTIER, J. (1969) L'anthracnose des cafeiers robusta et excelsa due a' Colletotrichum coffeanum Noack en République Centrafricaine. Cafe - Cacao - The 13 : 221 - 230.

117. SCHIEBER, E. , ZENTMEYER, G.A.& MITCHELL, D.J. ROHEIM, J.*(1970) Coffee berry necrosis in Guatemala and Costa Rica. Phytopathology 60 : 1542.

118. SHABI, E.& BEN-YEPHET, Y. (1976) Tolerance of Venturia pirina to benzimidazole fungicides. Plant Disease Reporter 60 : 451 - 454.

119. SHABI, E.& KATAN, T. (1979) Genetics, Pathogenicity and stability of carbendazim - resistant isolates of Venturia pirina. Phytopathology 69: 267 - 269.

120. SHALTA, M.N.& SINCLAIR, J.B. (1963) Tolerance to penachloronitrobenzene among cotton isolates of Rhizoctonia solani. Phytopathology 53: 1307 - 1411.

121. SIMMONDS, J.H. (1963) Studies on the latent phase of Colletotrichum causing ripe rots of tropical fruits. Queensland Journal of Agricultural and Animal Sciences 20 : 373 - 424.

122. SMALL, W. (1926) On the occurrence of a species of Colletotrichum. Trans. Brit. Mycol. Soc. 11 : 112 - 137.

123. SMILEY, R.W.& HOWARD, R.J. (1976) Tolerance to benzimidazole derivative fungicides by Fusarium roseum on Kentucky blue grassturf. Plant Disease Reporter 60 : 91 - 94.

124. SOZZI, D.& GESSLER, C. (1980) Fungicide (M B C) resistant mutants of Fusarium oxysporum f.sp. lycopersici and Botrytis cinerea : Pathogenicity and fitness. Phytopath. Z. 97 : 19 - 24.

125. STAPLES, D.C. (1973) An introduction to microbiology , 1st edition, MACMILLAN Education Ltd., London. 193 pp

126. STEINER, K.G. (1972) The influence of surface wax obtained from green berries of six selections of Coffea arabica on conidia of Colletotrichum coffeanum. Kenya Coffee 37 : 179.

127. SUTTON, T.B. (1978) Failure of combinations of benomyl with reduced rates of non-benzimidazole fungicides to control Venturia inaequalis resistant to benomyl and the spread of resistant strains in North Carolina. Plant Disease Reporter 62 : 830 - 834.

128. SZKOLNIK,M.& GILPATRICK, J.D. (1969) Apparent resistance of Venturia inaequalis to dodine in New York apple orchards. Plant Disease Reporter 53: 861 - 864.

129. TALBOYS, P.W. & DAVIES, M.K. (1976) Benomyl tolerance in Verticillium dahliae. Annals of Applied Biology 82 : 41 - 50.

130. TALBOYS, P.W.& DAVIES, M.K. (1976) Pathogenicity of benomyl tolerant variants of Verticillium dahliae to strawberry, CV. Cambridge Vigour. Annals of Applied Biology 83 : 489 - 493.

131. TALBOYS, P.W. ; FRICK, E.L.& DAVIES, M.K. (1975) Duration of effects of benomyl on growth and wilt (Verticillium dahliae) development in the strawberry, c.v. Cambridge Vigour, in relation to time of inoculation. Annals of Applied Biology 81 : 305 - 315.

132. TAPLEY, R.G. (1964) Coffee berry disease in Tanganyika. Tanganyika Coffee News 38 : 45.

133. TATE, K.G.& SAMUELS, G.J. (1976) Benzimidazole tolerance in Venturia inaequalis in New Zealand. Plant Disease Reporter 60 : 706 - 710.

134. TAYLOR, J. (1953) The effect of continual use of certain fungicides on Physalospora obtusa. Phytopathology 43 : 268 - 270.

135. THANASSOULOPOULOS, C.C. ; GIANNOPOLITIS, C.N. & KITSOS, G.T. (1971) Evaluation of sensitiveness and development of resistance of Fusarium oxysporum f. sp. lycopersici to benomyl. Phytopath. Z 70. 114 - 120.

136. THOROLD, C.A. (1945) Elgon dieback disease of coffee. East African Agricultural Journal 10 : 198 - 206.

137. van der VOSSEN, H.A.M. ; COOK, R.T.A.& MURAKARU, G.NW. (1976) Breeding for resistance to coffee berry disease caused by Colletotrichum coffeanum Noack (Sensu Hindorf) in Coffea arabica I. Methods of preselection for resistance. Euphytica 25: 733 - 745.

138. van der VOSSEN, H.A.M.& WALYARO, D.J. (1981) The coffee breeding programme in Kenya. A review of progress made since 1971 and plan of action for the coming years. Kenya Coffee 46 (No 541) : 113 - 130.

139. VERMEULEN, H. (1968) Screening of fungicides for the control of coffee berry disease in Kenya. Experimental Agriculture 4: 255 - 261.

140. VERMEULEN, H. (1970) Coffee berry disease in Kenya. II. The role of Glomerella cingulata in the Colletotrichum population colonising the bark of Coffea arabica. Neth. J. Plant Pathology 76 : 285 - 292.

141. VINE, B.H., VINE, P.A.& GRIFFITHS, E. (1973 a) Evaluation of fungicides for the control of coffee berry disease in Kenya. Annals of Applied Biology 75 : 359 - 375.

142. VINE, B.H., VINE, P.A.& GRIFFITHS, E. (1973 b) Some problems of evaluating fungicides for use on coffee in Kenya. Annals of Applied Biology 75 : 377 - 385.

143. von ARX, J.A. (1957) Die arten der gattung Colletotrichum Cda. Phytopath. Z. 2 : 413 - 468.

144. von ARX, J.A. (1970) A revision of the fungi classified as Gloeosporium. Lehre, Verlag J. Cramer.

145. WALLER, J.M. (1971) The incidence of climatic conditions favourable to coffee berry disease in Kenya. Experimental Agriculture 7 : 303 - 314.

146. WARREN, C.G., SANDERS, P.& COLE, H. (1974) Sclerotinia homeocarpa tolerance to benzimidazole configuration fungicides. Phytopathology 64 : 1139 - 1142.

147. WELLMAN, F.L. (1961) Coffee botany, cultivation and utilisation. London, Leonard Hill. 488 pp.

148. WHELLER, H.E. (1954) Genetics and evolution of heterothallism in Glomerella. Phytopathology 44: 342 - 345.

149. WHAN, J.H. (1976) Tolerance of Sclerotinia fructicola to benomyl. Plant Disease Reporter 60:200-201.

150. WICKS, T. (1974) Tolerance of the apple fungus to benzimidazole fungicides. Plant Disease Reporter 58:886 - 889.

151. WUEST, P.J., COLE, H. Jr. & SANDERS, P.L. (1974) Tolerance of Verticillium malthousei to benomyl Phytopathology 64 : 331 - 334.

Appendix I: Staining reagents:

(a) Farmers' fluid:

Absolute ethanol	400 ml
Glacial acetic acid	200 ml

(b) Lactophenol:

Distilled water	20 ml
Glycerine	20 ml
Lactic acid	20 ml
Phenol (Pure crystals)	20 g

(c) 0.1 Acid Fuchsin in Lactophenol:

95% ethanol	99 ml
Acid fuchsin (powder)	1 g

To 10 ml of the above, 90 ml of lactophenol was added.

Appendix 2: Preparation of Carnoy's fluid and Heidenhain's iron-alum haematoxylin solution:

(a) Carnoy's fluid:

Formula 6:3:1 (alcohol-chloroform - glacial acetic acid)

30 ml of chloroform was added to 60 ml of anhydrous ethyl alcohol and finally 10 ml of glacial acetic acid was added. To 95 ml of this solution 5 ml of NHCL was added.

(b) Heidenhain's iron-alum haematoxylin solution:

2 g of haematoxylin was dissolved in 100 ml 45% acetic acid (solution A)

0.5 g Ferric alum was in 45% acetic acid (solution B)

These fresh solutions A and B were mixed 1 day before use in a ratio of 1:1

Appendix 3: A list of media used for growth of Colletotrichum Coffeanum

1. Plain Agar:

Agar	6 g
Distilled water	400 ml.

2. Malt extract agar (MEA) (3.4%):

MEA	13.6 g
Distilled water	400 ml.

3.

Agar	8 g
Glucose	10 g
Potassium nitrate	0.8 g
Potassium phosphate	0.4 g
Magnesium sulphate	0.2 g
Zinc sulphate	0.004 g
Ferrous sulphate	0.004 g
Copper sulphate	0.0004 g
Distilled water	400 ml.

4.

Agar	8 g
Sucrose	20 g
Potassium nitrate	0.8 g
Potassium phosphate	0.4 g
Magnesium sulphate	0.2 g
Zinc sulphate	0.004 g
Ferrous sulphate	0.004 g
Copper sulphate	0.0004 g
Distilled water	400 ml.

5.	Agar	8 g
	Starch	30 g
	Potassium nitrate	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
6.	Agar	8 g
	Cellulose	30 g
	Potassium nitrate	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
7.	Agar	8 g
	Glucose	10 g
	Ammonium nitrate	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
8.	Agar	8 g
	Sucrose	20 g
	Ammonium nitrate	0.8 g

	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
9.	Agar	8 g
	Starch	30 g
	Ammonium nitrate	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
10.	Agar	8 g
	Cellulose	30 g
	Ammonium nitrate	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
11.	Agar	8 g
	Glucose	10 g
	Peptone	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g

	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml
12.	Agar	8 g
	Sucrose	20 g
	Peptone	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.
13.	Agar	8 g
	Starch	30 g
	Peptone	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.004 g
	Distilled water	400 ml.
14.	Agar	8 g
	Cellulose	30 g
	Peptone	0.8 g
	Potassium phosphate	0.4 g
	Magnesium sulphate	0.2 g
	Zinc sulphate	0.004 g
	Ferrous sulphate	0.004 g
	Copper sulphate	0.0004 g
	Distilled water	400 ml.

Similar media were prepared from numbers 3 to 14 but these media were without zinc sulphate, ferrous sulphate and copper sulphate.

15. Cherry agar:

The extract:

½ kg of ripe cherry of SL 28 coffee variety was surface sterilized for 5 minutes using 1% calcium hypochlorite then washed in 5 changes of distilled sterile water and then put in a Waring blendor. 1000 ml distilled water was added and the cherry was macerated thoroughly. The resulting mixture was expressed through cheese cloth. The expressed extract was retained while the pulp and bean remains were discarded. The extract was sterilized at 110°C for 1 hour.

The medium:

Cherry extract	120 ml
Distilled water	280 ml
Agar	8 g.

Appendix 4: The effect of temperature on pathogenicity of sensitive (S) and tolerant (T_1 and T_2) strains to detached berries of SL 28 coffee cultivar at the 25th day from inoculation:

ANOVAR TABLE

	SS	df	MS	F Value	
Strains	91.8222	2	45.9111	1.7352	N.S
Temp. (T)	140672.1777	4	35168.0444	1329.1424***	
Days (D)	226855.6444	7	32407.9492	1224.8273***	
I x T	1979.2888	8	247.4111	9.3507***	
I x D	440.0888	14	31.4349	1.1881	N.S
T x D	53181.6888	28	1899.3460	71.7840***	
Pooled Error	7831.9112	296	26.4592		
Total	431052.6222	359			

Appendix 5: The effect of conidial concentration of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum on detached berry infection of SL 28 coffee cultivar at the 25th day from inoculation:

ANOVAR TABLE

	SS	df	MS	F Value
Strains	4667.9110	2	2333.9555	22.7357***
Days (D)	289050.3109	7	41292.9015	402.2454***
Spore Conc. (S.C.)	365831.4662	9	40647.9406	395.9626***
Strains x D	6016.8889	14	429.7777	4.1866***
Strains x S.C.	10257.8658	18	569.8814	5.5514***
D x S.C.	133788.7987	63	2123.6317	20.6869***
Strains x D x S.C.	12934.6652	126	102.6560	
Error	5066.6711	480	10.5555	
Total	827614.5778	719		

Appendix 6: Pathogenicity of benomyl sensitive and tolerant strains of C. coffeanum to detached berries of SL 28 in the presence of benomyl:

(a) Table of means (strains x ben. Concs)

Benomyl Concs (µg/ml)	CBD S	Strains T_1	T_2	Benomyl Conc. Means
0	67.75	60.75	54.75	61.08
0.25	50.58	61.08	54.08	55.25
0.5	42.50	61.00	55.50	53.00
1	39.58	60.08	54.00	51.22
2	35.67	57.58	49.33	47.19
2.5	43.92	68.25	58.25	56.81
5	39.67	69.08	55.00	54.58
10	38.67	66.33	52.17	52.39
25	35.00	61.08	47.08	47.72
50	25.33	56.42	43.42	41.72
100	24.75	38.58	41.75	35.03
125	23.00	38.92	41.50	34.47
250	14.67	37.00	37.33	29.67
500	14.67	30.67	29.58	24.97
1000	12.25	31.42	28.33	24.00
Strain Means	33.87	53.15	46.81	

	5% level	1% level
L S D (Strains)	1.82	2.39
L S D (Ben. Conc.)	4.07	5.35

Appendix 6:

(b) Table of means (Days of recording x ben.Concs)

Benomyl Conc(µg/ml)	Days of recording (D) D4	D7	D10	D13	D16	D19	D22	D25	Benomyl Conc. Means
0	0.00	16.67	52.67	65.78	78.67	84.22	94.00	96.67	61.08
0.25	0.00	12.22	38.22	54.44	69.78	81.56	89.11	96.67	55.25
0.5	0.00	9.56	35.78	54.89	68.44	78.22	87.11	90.00	53.00
1	0.00	9.78	32.67	49.56	64.44	76.67	86.00	90.67	51.22
2	0.00	8.00	24.89	45.11	57.11	71.11	82.89	88.44	47.19
2.5	0.00	11.56	52.44	66.22	73.56	80.67	84.22	85.78	56.81
5	0.00	10.44	50.89	62.44	69.56	78.22	81.11	84.00	54.58
10	0.00	8.67	46.00	59.78	67.33	76.00	79.33	82.00	52.39
25	0.00	7.33	40.00	50.44	60.22	70.89	74.67	78.22	47.72
50	0.00	5.33	34.67	45.33	53.11	60.22	65.33	69.78	41.72
100	0.00	2.00	21.56	36.89	46.89	52.44	57.78	62.67	35.03
125	0.00	1.33	20.89	34.22	46.00	53.33	57.78	62.22	34.47
250	0.00	0.00	16.00	27.78	41.11	46.00	52.00	54.44	29.67
500	0.00	0.00	11.56	24.00	31.11	38.22	45.11	49.78	24.97
1000	0.00	0.00	11.33	24.00	31.11	36.67	42.00	46.89	24.00
Day Means	0.0	6.86	32.64	46.73	57.23	65.63	71.90	75.88	

	5% level	1% level
L S D (Days)	2.97	3.91
LSD(Ben.Conc)	4.07	5.35

Appendix 6: Pathogenicity of benomyl sensitive and tolerant strains of C. coffeanum to detached berries of SL 28 in the presence of benomyl:

(c) Table of means -- % infection (benomyl conc. x unpuctured/puctured berries):

Benomyl Conc.-µg/ml	Berries U	Berries P	Ben.Conc. Means
0	51.83	70.33	61.08
0.25	43.50	67.00	55.25
0.5	42.06	63.94	53.00
1	40.28	62.17	51.22
2	38.22	56.17	47.19
2.5	40. 61	73.00	56.81
5	38.78	70.39	53.58
10	35.83	68.94	52.39
25	28.33	67.11	47.72
50	21.33	62.11	41.72
100	17.22	52.83	35.03
125	13.61	55.33	34.47
250	10.83	48.50	29.67
500	6.50	43.44	24.97
1000	3.56	44.44	24.00
U/P Means	28.83	60.28	

	5% level	1% level
L S D (U/P)	1.49	1.96
L S D (Ben.Conc.)	4.07	5.35

Appendix 6 Pathogenicity of benomyl sensitive and tolerant strains of *C. coffeanum* to detached berries of SL 28 in the presence of benomyl:

(d) Table of means (Days of recording x Unpuctured/puctured berries)

Berries	Days of recording (D) D4	D7	D10	D13	D16	D19	D22	D25	U/P Means
Unpuctured (U)	0.00	2.28	14.37	24.27	34.79	44.59	52.47	57.90	28.83
Puctured (P)	0.00	11.44	50.90	69.19	79.67	86.67	91.32	93.87	60.38
D Means	0.00	6.86	32.64	46.73	57.23	65.63	71.90	75.88	

	5% level	1% level
L S D (U/P)	1.49	1.96
L D S (D)	2.97	3.91

Appendix 7: The weather conditions (temperature relative humidity (R.H) and rainfall) at the time when field inoculations were carried out at CRS, Ruiru:

(a) Month: June						(b) July				
	Temperature (°c)		R. H.		Rainfall	Temperature (°c)		R.H.		Rainfall
Dates	max.	min.	M.	A	(mm)	max.	min.	M	A	(mm)
1		13.5	97	72			8.3	96	77	
2	22.6	13.8	88	69	0.3	22.0	10.0	93	72	
3	24.2	14.2	98	71	3.1	23.4	7.2	86	67	
4	23.3	14.2	91	81	1.4	23.3	7.5	82	75	
5	23.4	12.8	97	92	0.2	22.5	13.4	86	61	
6	23.3	14.6	96	79		24.7	12.2	96	76	
7	23.4	15.2	83	82		20.6	13.3	87	81	
8	24.4	14.6	93	66		23.2	11.4	83	58	
9	24.3	14.7	93	80		26.4	2.5	78	78	
10	24.7	13.7	90	78	0.5	23.7	13.4	90	71	
11	23.0	14.5	95	76	0.5	23.2	13.5	84	96	
12	23.3	11.0	84	61		21.2	14.0	94	81	5.6
13	24.7	9.4	79	58		22.7	14.3	97	98	3.5
14	24.6	9.0	79	78		22.4	14.1	91	80	1.1
15	24.5	12.3	83	64		23.4	13.6	96	84	0.4
16	25.7	9.4	95	73		21.3	13.4	92	98	Tr
17	25.8	14.1	94	83		20.3	10.7	98	65	
18	23.8	14.4	94	75	0.1	25.3	14.0	98	96	
19	24.1	12.5	79	69		6.2	2.8	94	84	0.3
20	23.0	12.1	81	65		20.2	11.2	92	86	0.3
21	24.5	8.6	91	72		7.8	10.4	94	75	
22	24.8	10.8	83	81		19.5	9.6	90	71	
23	25.8	10.9	91	65		19.1	12.0	90	66	
24	26.3	14.7	88	80		20.4	8.0	84	66	
25	23.8	12.0	87	71		25.7	5.8	86	64	
26	23.9	11.7	75	66		23.4	11.8	81	69	
27	24.3	11.4	98	83	Tr	23.3	13.0	91	79	
28	22.5	9.9	93	87	Tr	19.7	13.1	92	73	
29	22.7	14.2	86	69		20.1	8.2	85	74	
30	25.3	13.2	87	77		24.8	10.5	78	83	
31						23.2	11.8	87	74	

Appendix 8 a: Radial mycelial growth of strains S, T_1 and T_2 of C. coffeanum on various formulated media:

Table of means (mm)

Media	Strains S	Strains T_1	Strains T_2	Media Means
1	29.17	30.00	39.50	32.89
2	77.33	79.00	78.67	78.33
3	39.00	66.50	65.33	56.94
4	55.67	66.67	70.50	64.28
5	42.83	56.67	17.33	38.94
6	40.17	20.83	30.83	30.61
7	30.50	37.83	36.67	35.00
8	37.67	36.17	40.33	38.06
9	36.17	22.50	20.17	26.28
10	30.00	30.83	30.83	30.56
11	66.50	77.67	66.67	70.28
12	73.67	82.67	83.67	80.00
13	77.83	75.83	77.83	77.17
14	56.00	50.83	54.50	53.78
15	73.83	78.67	80.33	77.61
16	38.83	66.50	65.00	56.78
17	78.67	75.17	71.00	74.94
18	48.83	56.17	62.00	55.67
19	49.67	42.83	46.83	46.44
20	26.33	44.83	46.17	39.11
21	22.67	31.83	29.83	28.11
22	60.00	58.67	60.33	59.67
23	60.50	61.17	59.17	60.28
24	74.00	83.00	77.33	78.11
25	78.17	83.17	83.83	81.72
26	67.83	52.33	65.67	61.94
27	54.50	38.83	51.50	48.28
Strain Means	52.83	55.82	55.99	

	5% level	1% level
L S D (Strains)	0.54	0.71
L S D (Media)	1.62	2.13

Appendix 8 b: Radial mycelial growth of strains S, T_1 and T_2 of C. coffeanum on different formulated media:

ANOVAR TABLE

	SS	df	MS	F Value
Strains	513.5576	2	256.7788	83.3914***
Medium	79099.7056	26	3042.2963	988.0152***
Strain x Medium	9340.9423	52	179.6335	58.3377***
Within error	498.8335	162	3.0792	
Total	89453.0391	242		

Appendix 9: Influence of carbon, nitrogen and heavy metals on radial mycelial growth of strains S, T_1 and T_2 of C. coffeanum:

ANOVAR TABLE

Source of variations	SS	df	MS	F	P
Strains	138.4668	2	69.2334	0.7219	N S
Carbon Source	2473.8086	3	824.6029	8.5979	***
Nitrogen Source	10308.8965	2	5154.4483	53.7441	***
Cations	993.7625	1	993.7625	10.3617	**
S x C	1184.8106	6	197.4684	2.0590	N S
S x N	124.2938	4	31.0735	0.3240	N S
S x Ct	76.2696	2	38.1348	0.3976	N S
C x N	4234.0914	6	705.6819	7.3580	***
C x Ct	489.5779	3	163.1926	1.7016	N S
N x Ct	978,4983	2	489.2492	5.1013	*
Error	3836.2878	40	95.9072		
Total	24838.7638	71			

Appendix 10 a: Sporulating capacity of strains S, T_1 and T_2 of C. coffeanum on different formulated media:

Table of means (conidia/mm^2) x 1000

Media	Strains S	T_1	T_2	Media Means
1	0.52	0.85	0.52	0.63
2	25.27	31.62	35.03	30.64
3	24.29	32.28	36.60	31.05
4	25.14	36.14	39.15	33.48
5	23.83	14.99	21.21	20.01
6	4.78	3.80	4.13	4.23
7	13.62	8.97	10.34	10.98
8	12.64	9.49	12.05	11.39
9	9.89	6.02	8.38	8.10
10	3.86	3.27	3.34	3.49
11	31.43	36.21	36.01	34.55
12	28.09	52.83	39.67	40.20
13	21.47	33.78	29.27	28.17
14	8.64	5.30	9.56	7.84
15	15.45	13.55	14.60	14.53
16	15.84	24.22	23.44	21.17
17	23.44	24.29	25.47	24.40
18	20.69	13.68	15.65	16.67
19	5.43	3.60	3.80	4.28
20	14.47	19.58	21.80	18.62
21	19.58	19.77	20.23	19.86
22	11.92	13.49	11.92	12.44
23	5.31	0.72	2.36	2.79
24	27.24	30.05	36.60	31.29
25	30.25	29.20	39.02	32.82
26	14.34	17.55	31.88	21.26
27	6.61	8.05	3.60	6.09
Strain Means	16.45	18.27	19.84	

	5% level	1% level
L S D (Strains)	0.14	0.18
L S D (Media)	0.41	0.54

Appendix 10 b: Sporulating capacity of strains S, T_1 and T_2 of *C. coffeanum* on different formulated media:

ANOVAR TABLE

	SS	df	MS	F Value
Strains	466.9871	2	233.4935	1237.3794***
Media	31561.9994	26	1213.9230	6433.0843***
Strains x media	2881.5823	52	55.4150	293.6672***
Pooled error	30.5782	162	0.1887	
Total	34941.1471	242		

Appendix II : Sporulating capacity of strains S, T_1 and T_2 of C. coffeanum on different formulated media:

ANOVAR TABLE

Source of variation	SS	df	MS	F	P
Strains	142.6583	2	71.3292	4.0071	*
Carbon Source	5375.0972	3	1791.6991	100.6527	***
Nitrogen Source	2486.8221	2	1243.4111	69.8514	***
Cations	59.3505	1	59.3505	3.3341	*
S x C	157.5562	6	26.2594	1,4752	N S
S x N	135.4991	4	33.8748	1,9030	N S
S x Ct	18.2923	2	9.1462	0.5138	N S
C x N	639.6922	6	106.6154	5.9894	***
C x Ct	7.9643	3	2.6548	0.1491	N S
N x Ct	412.3278	2	206.1639	11.5817	***
Error	712.0328	40	17.8008		
Total	10147.2928	71			

Appendix 12: The effect of glucose as external source of carbon on percent germination of conidia of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum on glass surfaces:

ANOVAR TABLE

	SS	df	MS	F Value
Strains	603.4276	2	301.7138	336.246***
Glucose conc.	15340.1365	6	2556.6894	2849.314***
StrainsxGlucose conc.	1962.3392	12	163.5282	182.245***
Error	37.6900	42	0.8973	
Total	17943.5935	62		

Appendix 13: The effect of benomyl on conidial germination of strains S, T_1 and T_2 of *C. coffeanum*

Table of means (% germination)

Strain (1)	Hours of incubation (Hrs)	Benomyl concentration (F.C.) - µg/ml 0	0.25	0.5	1	2	2.5	5	10	25	50	100	125	250	500	1000	Means (1XHrs)	Means Strain
S	8	13.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	
	24	36.5	3.8	0.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	
	48	54.5	13.6	4.9	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	
	72	78.5	22.9	7.4	6.5	2.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.9	
Means(1XF.C)		45.7	10.2	3.3	2.4	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		4.2
T_1	8	1.6	1.8	1.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	
	24	3.0	2.7	2.5	1.9	1.5	1.2	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	
	48	19.8	19.8	18.4	16.7	15.1	16.2	13.2	12.8	7.6	5.8	2.8	2.3	0.7	0.0	0.0	10.1	
	72	30.2	30.8	29.2	28.1	26.2	25.4	24.0	21.7	19.8	17.2	8.3	4.1	1.0	0.0	0.0	17.7	
Means(1XF.C)		13.6	13.8	12.9	11.9	10.7	10.7	9.5	8.7	6.9	5.8	2.8	1.6	0.4	0.0	0.0		7.3
T_2	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	24	2.0	2.0	1.7	1.8	1.3	1.7	1.5	1.3	0.9	0.8	0.0	0.0	0.0	0.0	0.0	1.0	
	48	13.0	14.1	12.6	11.2	11.3	13.5	12.1	11.8	10.1	6.9	2.3	1.3	0.8	0.0	0.0	8.1	
	72	23.0	24.5	23.2	21.5	20.9	23.2	22.0	20.8	20.6	16.8	5.3	3.0	1.8	0.0	0.0	15.1	
Means(1XF.C)		9.5	10.2	9.4	8.6	8.4	9.6	8.9	8.5	7.9	6.1	2.1	1.1	0.6	0.0	0.0		6.1
Means F.C.XHrs	8	4.9	0.9	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		Mean(Hrs) 0.4
	24	13.8	2.8	1.7	1.3	0.9	1.0	0.7	0.6	0.3	0.3	0.0	0.0	0.0	0.0	0.0		1.6
	48	29.2	15.8	12.0	10.2	8.8	9.9	8.6	8.2	5.9	4.2	1.9	1.2	0.5	0.0	0.0		7.8
	72	43.9	26.1	19.9	18.7	16.5	16.5	15.3	14.2	13.5	11.3	4.6	2.4	0.9	0.0	0.0		13.6
Mean (F.C.)		22.9	11.4	8.5	7.6	6.6	6.8	6.1	5.7	4.9	4.0	1.6	0.9	0.4	0.0	0.0		

	5% level	1% level
LSD(strains)	0.11	0.15
LSD(Hrs)	0.13	0.17
LSD(F.C.)	0.25	0.32
LSD(1XHrs)	0.22	0.29
LSD(F.C.XHrs)	0.49	0.65
LSD(1XF.C.)	0.43	0.56

Appendix 14: The effect of temperature on appressorial formation of strains S, T_1 and T_2 of C. coffeanum:

Table of means (% appressorial formation)

Strain (1)	Temp. (T) °C	Hours of incubation (H) 8	24	48	72	Means 1 x T.	Means strain
S	5	0.0	0.0	1.4	7.4	2.2	
	10	0.6	4.5	11.4	17.8	8.8	
	15	2.3	10.0	19.2	27.2	14.7	
	20	24.1	35.4	43.7	65.3	42.1	
	25	20.4	35.1	54.6	60.6	42.7	
	30	5.9	12.1	30.4	31.3	19.9	
							21.7
Means (1xH)		9.1	16.2	26.8	34.9		
T_1	5	0.0	0.0	0.0	0.8	0.2	
	10	0.0	3.5	6.4	8.6	4.6	
	15	0.0	12.3	17.6	21.4	12.8	
	20	7.9	14.5	20.1	29.4	18.0	
	25	4.8	15.3	24.4	31.3	19.0	
	30	0.4	3.1	10.4	11.3	6.3	
							10.1
Means (1xH)		2.2	8.1	13.2	17.1		
T_2	5	0.0	0.0	0.0	3.5	0.9	
	10	0.0	1.9	10.7	15.8	7.1	
	15	0.0	6.4	15.4	29.9	12.9	
	20	0.0	17.7	24.8	27.2	17.4	
	25	0.0	8.9	14.8	17.5	10.3	
	30	0.0	4.2	6.8	11.0	5.5	
							9.0
Means (IxH)		0.0	6.5	12.1	17.5		
							Means (T)
Means TxH	5	0.0	0.0	0.5	3.9		1.1
	10	0.6	3.3	9.5	14.1		6.9
	15	0.8	9.5	17.4	26.2		13.5
	20	10.7	22.5	29.5	40.6		25.8
	25	8.4	19.8	31.3	36.4		24.0
	30	2.1	6.5	15.8	17.9		10.6
Means (H)		3.8	10.3	17.3	23.2		

	5% level	1% level
L S D (Strains)	1.9	2.5
L S D (Hours)	2.2	2.9
L S D (Temps)	2.7	3.5
L S D (1xH)	3.8	4.9
L S D (IxT)	4.6	6.1
L S D (HxT)	5.3	7.0

Appendix 15: The effect of benomyl on mycelial growth of sensitive (S) and tolerant (T_1 and T_2) strains of C. coffeanum:

A N O V A R T A B L E

	SS	df	MS	F Value
Reps	319.1027	2	159.5514	14.4990
Strains	58157.5897	2	29078.7948	2642.5017***
Benomyl Conc.	44265.4756	14	3161.8196	287.3266***
Strains x Ben. Conc.	14283.8802	28	510.1385	46.3582***
Error	968.3755	88	11.0042	
Total	117994.4240	134		