FUNGI ASSOCIATED WITH THE NEW MALADY OF COFFEE IN SOUTH INDIA*

By V. Agnihothrudu, F.A.Sc.

(Technical Adviser, Rallis India Limited, 6 A, Cunningham Road, Bangalore-1 B)

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Abstraot

A marked decline in the coffee yields was first noticed in Mysore during the monsoon period of 1957. The leading symptom which manifests between March/May was found to be chlorosis and epinasty of penultimate or sub-penultimate pair of leaves followed by their death and the extension of necrosis from the leaf scar into the internodes. Occasionally, browning of the pith and intercellular mycelium was noticed. Other symptoms attributed to the New Malady are the die-back of productive branches, crinkling of leaves, witches' broom-like growth, shortening of internodes, etc.

In soil mycological studies, 48 species of fungi were isolated in this laboratory of which the following are potential pathogens:

Botryodiplodia theobromae Pat., *Fusarium semitectum* Berk. *et* Br., *F. oxysporum* Schlecht., *F. solani* (Mart.) App. *et* Wr., *F. stilboides* Wr. *Hypomyces* sp. and *Nectria haematococca* Berk. *et* Br.

In the aerial parts, *Colletotrichum coffeanum* Noack was the most frequent isolate. This fungus was present on apparently healthy plants as a part of the surface mycoflora. The perfect stage of this fungus, *Glomerella cingulata* (Stonem.) Spauld. *et* Schrenk. was also isolated though it was not common. *Colletotrichum coffeanum* was found associated with lesions on leaves, berries and twigs. A total of 87 species of fungi was isolated from different sources, 20 of which are potential pathogens. While the etiology of the Malady is still obscure, the evidence we have, indicates that *Colletotrichum coffeanum* Noack may be the fungus involved in the disease.

There is heavy sporulation of *Colletotrichum* with the first showers in April/May period and in order to reduce the initial inoculum load, a pre-blossom spray in March is now recommended with encouraging results.

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FUNGI ASSOCIATED WITH THE NEW MALADY OF COFFEE IN SOUTH INDIA

A peculiar decline of coffee which was first noticed during the monsoon of 1957 in South and West Mysore assumed serious proportions by early 1959 with the wide spread die-back and die-forward of growing branches, following blossom showers. The disease was found to be marked in old stands of Arabica coffee. The Balehonnur station selections were also affected, but, not to the same degree as the old Kents.

The leading symptoms of the malady as observed by us in the last 7 years (Agnihothrudu, 1964 *a*, *b*, *c*; 1965, *a*, *b*, *c*, *d*; and 1966) are: The growing buds or terminal leaves generally on the tertiary and at times on the secondary branches show necrosis. In several instances the penultimate or the subpenultimate pair of leaves are shed and the necrosis extends from the leaf scar. Very often when the subpenultimate pair of leaves are affected, the terminal and penultimate leaves show chlorosis and epinasty. Occasionally, the bushes are affected only on one side, the other side(s) remaining apparently unaffected. Sometimes a fungal mycelium was noticed in the cambial region accompanying the brown discolouration in vessels, tracheids and xylem parenchyma. Besides the above, (1) goose-neck like growth of tertiary branches, degrees, (2) bluish-green coriaceous foliage, (3) leaf chlorosis of different, (4) witches' broom growth, (5) shortening of inter-nodes, (6) rosetting of leaves were observed. It is apparent that a whole gamut of symptoms has been clubbed under the New Malady. In order to sort out the individual groups of symptoms and establish the etiology, we have chosen 27 estates in Coorg, Saklespur and Hassan zones with investigation blocks (Agnihothrudu, 1968).

Soil Mycological Studies

As one of the phases of investigation, soil biological studies were conducted both in the soil away from roots and in the rhizosphere region also (Agnihothrudu, 1965 *b*, 1965 *c*); Two species of *Fusarium*, belonging to the *Elegans* and *Martiella*, the mycelia of which were intercellular in the dead roots and collar of the plants were encountered. Over 130 soil samples were analysed using Warcup (1950) method and for root fungi Agnihothrudu's (1953) method. The total number of fungal species recorded was 48 out of which the following are potential pathogens: *Botryodiplodia theobromae* Pat., *Fusarium semitectum* Berk. *et* Rav., *F. oxysporum* Schelcht., *F. solani* (Mart.) App. *et* Wr., *F. stilboides* Wr. (= *F. lateritium* Nees.), *Hypomyces* sp. and *Nectria haematococca* Berk. *et* Br.

In several instances on the roots of up-rooted plants rhizomorphs of a fungus were seen. These were observed on apparently healthy plants as well as on plants showing signs of die-back. This is most probably the vegetative phase of some saprophytic basidiomycete. Amongst other fungi, 5 isolates of *Mycelia sterilia* were isolated. They were not conclusively identified.

Fungi Associated with the Aerial Parts

Samples collected during nearly 200 visits to coffee estates in Coorg, Hassan and Chikmagalur Districts were planted on nutrient agar, and the fungi were isolated from bark, twigs, leaves and berries of coffee and soils from the estates under intensive observation. The fungi isolated from different sources are:

Absidia spinosa Lendner, *Acrothecium* sp., *Arthrobotrys superba* (Corda) Sacc., *Ascochyta* sp., *Alternaria humicola* Oud., *Alternaria* sp., *Amphitiarospora neottiosporoides* Agni., *Aspergillus candidus* Link., *A. fischeri* Wehner, *A. flavus* Link., *A. fumigatus* Fres., *A. nidulans* (Edam) Winter, *A. niger* van Tieghem, *A. tamarii* Kita, *A. ustus* (Bainier) Thom *et* Church, *Bispora* sp., * *Botryodiplodia theobromae* Pat., * *Botrytis cinerea* Fr., *Botryosporium longibrachiatum* Carda, * *Capnodium brasiliense* Puttem., *Cephalosporium* sp., * *Cercospora coffeicola* Berk. *et* Cooke, * *Cercospora* sp., *Chaetomium globosum* Kunze, *C. indicum* Corde, *Chaetophoma* sp., *Chaetospermum* sp., *Circinella spinosa* van Tieghem *et* Le Monnier, *Cladosporium herbarum* Link, * *Colletotrichum coffeanum* Noack, *Cunninghanella bertholletiae* Stadel, *C. echinulata* Thaxter, *Curvularia lunata* (Wakker) Boedijn, *C. maculans* (Bancroft) Boedijn, *C. pallescens* Boedijn, *C. trifolli* (Kauf.) Boedijn *sensu* Parmelee, *Cylindrocarpon tenue* Bugincourt, * *Cylindrocladium scoparium* Morg., *Dendryphiopsis interseminata* (Berk. *et* Rav.) Hughes, *Didymostilbe coffeae* P. Hann., *Fusarium equiseti* (Corda) Sacc. *F. Gibbosum* App. *et* Wr., * *F. semitectum* Berk. *et* Rav., * *F. oxysporum* Schlecht., * *F. solani* (Mart.) App. *et* Wr., * *F. stilboides* Wr., (= *F. lateritium* Nees.), *Gliocladium roseum* (Link?) Bainier, * *Glomerella cingulata* (Stonem.) Spauld. *et* v. Schrenk, *Helminthosporium* sp., *Haplosporella* sp., * *Hemileia vastatrix* Berk. *et* Br., *Heterosporium terrestre* Atk., *Hormodendrum resinae* Lindau, * *Hypomyces* sp., *Kutilakesopsis macalpineae* Agni.,

* Potential Pathogens.

Melanospora sp., *Mucor* sp., *M. racemosus* Fr., * *Mycosphaerella coffeicola* Cke., *Mycotypha dichotoma* Wolf, * *Nectria haematococca* Berk. *et* Br., * *Neocosmospora vasinfecta* E.F.S., *Nigrospora oryzae* (B. *et* Br.) Petch, *Papularia* sp., * *Pellicularia filamentosa* (Pat.) Rogers (= *Koleroga knoxia* Donk.), *Penicillium* sp., *Pestalotiopsis* sp., *Phoma* sp., *Pullularia pullulans* (de Bary *et* Low) Berkhout, *Pyrenochaeta* sp., * *Rhizoctonia bataticola* (Taub.) Butler, *Rhizopus arrhizus* Fischer, *R. nodosus* Namyslowski, *Scopulariopsis* sp., *Sphaeronema* sp., *Spicularia terrestris* Timonin, *Sporotrichum roseum* Link, *Stachybotrys atra* Corda, *Stachylidium bicolor* Link *et* Fr., *Syncephalastrum racemosum* Schroet., * *Thielaviopsis basicola* (Berk. *et* Br.) Ferraris, *Thozetellopsis tocklaiensis* Agni., *Trichoderma viride* Fers. *ex* Fr., *Verticillium* sp., *V. hemilieae* Bouriquet ?

In Table I are presented, the number of samples analysed, and the total number of species of fungi isolated from each source.

A total of 87 species of fungi was isolated from different sources. Of them nearly 20 are potential pathogens. This is perhaps the first attempt at an exhaustive study of mycoflora associated with die-back of coffee in South India.

TABLE I

Analysis of samples of fungi identified

Source	Number of samples analysed	Total number of species of fungi	Number of potential pathogens
Bark and twigs ..	14520	59	14
Roots ...	172	52	11
Leaves ...	785	37	12
Fruit ...	420	31	15
Soil ..	130	48	7

Of all the isolates of fungi *Colletotrichum* was the most ubiquitous and this agrees remarkably well with the authenticated coffee pathogen, *viz.*, *Colletotrichum coffeanum* Noack deposited in Centralbureau Voor Schimmel-

cultures in Holland and Commonwealth Mycological Institute in London. 86% of twigs and bark, 56% of leaves and 89% of fruit yielded *C. coffeanum*. As nearly 15,000 samples which were screened for the fungal flora were mostly from dead or moribund bushes and bushes which were showing all visual symptoms of the New Malady, further investigations were made to find out whether *Colletotrichum* was merely one of the components of surface mycoflora. With this end in view, twigs, lamina, petiole from coffee bushes which were apparently healthy and dead were chosen and the plant parts plated on potato dextrose agar. The specimens were divided into two lots. One lot was subjected to surface sterilizing agent and the other lot was washed in repeated changes of sterile distilled water. In the case of twigs two separate samples were analysed. In one sample only internodal part and the other nodal part with the leaf scar were included. The results which are based on a study of 200 samples are presented in Table II. In the twig the internodal part from apparently healthy plants did not yield *Colletotrichum*, whereas the specimens obtained from dead twigs nearly 14% yielded the fungus. On the other hand the nodal part whether from dead or apparently healthy plants, surface sterilized or not, yielded *Colletotrichum*. The percentage, however, was very high in dead twigs (78%), indicating that the fungus is present within the plant tissues. The lamina and petiole not surface sterilized yielded

TABLE II

Percentage occurrence of Colletotrichum coffeanum *in plant parts, both surface sterilised and surface not sterilised*

	Dead plants	Plant parts apparently healthy	
	Surface sterilized	Surface sterilized	Surface not sterilized
Root ...	Nil	Nil	Nil
Twig (internode) ..	14	Nil	22
Twig (node) ...	78	3	19
Lamina ...	18	Nil	38
Petiole ...	64	Nil	27

Colletotrichum indicating that the fungus is a common component of the phyllosphere.

We have compared several isolates of *Colletotrichum coffeanum*. There were differences amongst sizes of conidia, length of setae, etc. There seems to be other variations, but, they are likely to be so under different conditions of growth and climate (Table III). In the course of our investigations *Colleto-*

TABLE III

Morphological variations in isolates of Colletotrichum coffeanum *grown on potato dextrose agar*

Isolate	Size of conidia	Measurement of setae	Type of growth	Colour on the reverse
Cannancadoo ..	10—13 (—14) × 2—3 (—4)	60—72 (—75)	Effuse	Olive
Karadibetta ..	8—12 (—15) × 2—4 (—5)	62—80 (—85)	Effuse	Pale Brown
Ossoor ..	10—12 (—14) × 3—4 (—5)	70—75 (—80)	Sparse cottony	Deep Olive
*Wartyhully ..	12—14 (—16) × 3—4 (—5)	65—70 (—85)	Dense cottony	Olive
Lingapur ..	14—16 (—18) × 3—4 (—6)	70–80 (—102)	Thin and adpressed	Deep Brown
*Biccode ..	12—15 (—16) × 2—3 (—4)	56—64 (— 70)	Effuse	Olive
Arabidacool ..	14—18 (—20) ×3—4 (—6)	75—85 (—120)	Thick cottony	Light Olive
Bettadamane ..	18—25 (—28) × 3—5 (—6·5)	80—95 (—130)	Effuse	Dilute Brown
*Emmekhan ..	14—16 (—18) × 2—3 (—5)	80—90 (—110)	Cottony	Dark Olive
Santaverry ..	10—12 (—14) × 2—4 (—5)	60—70 (—85)	Ropy	Dark Olive

* In these isolates perithecial production was noticed in the medium. The measurements are in microns.

trichum coffeanum was seen associated with *Hemileia* spots invading the necrotic tissues, as perhaps one of the members of an ecological succession of fungi, but, in many others it was within the tissues, irrespective of any primary infection by leaf disease or by any other parasite.

Colletotrichum coffeanum *and its Occurrence*

On leaves.—As a primary pathogen on the leaves the spots produced by this fungus are at first brown, then grey, irregular or roundish, those at the margin being elongated and are generally limited by the main nerves of leaf. Some of the spots are upto 3 cm in diameter and the edge is not bounded by a dark band as in the case of spots produced by *Mycosphaerella coffeicola* Cooke. The acervuli are visible as small black pustules on the upper surface of the leaf, produced in concentric rings.

In some instances, the fungus is associated with *Hemileia* spots. The *Hemileia* spots on the leaf are generally attacked by *Verticillium hemileiae* Bouriquet (*nomen nudum*) which in its turn is invaded by *Colletotrichum* (Hyperparasitism?). In several samples, species of *Pestalotiopsis* Steyaert were also seen, perhaps as one of the members of tertiary stages of succession. Although obvious damage is caused to leaves by *Colletotrichum coffeanum* the economic losses from this disease are difficult to assess particularly when association of this fungus with weak spotting and premature leaf fall are considered..

On berries.—The spots on berries are more delimited. Sometimes, the acervular setae appear on the edge of the fructification and they are well developed. The effect of the fungus is to form a shallow depression or to check the growth of the berry on one side, making it lop-sided, occasionally mummifying it. Fortunately, this form of attack appears to be uncommon in the estates under our observation. The associated organism is in no way different from the one causing Coffee Berry Disease in East Africa. It may be necessary to institute a thorough survey into the occurrence of this disease in coffee plantation in Southern India. In several instances the disease was anthracnose or brown blight (*sensu* Hocking, 1966) mostly latent. By latent it is meant infections which are contained (viable) by defensive host reactions but later becoming active either due to physiological imbalances or environmental conditions or both.

On twigs:—The fungus produces elongated, sharply defined spots surrounded by a slightly elevated margin. The interpetiolar stipules are blackened. It is a general observation that affected plants have shed most of their

leaves. Whether defoliation is the cause or effect needs careful investigation.

The fungus occurring in Mysore has been confirmed to be *Colletotrichum coffeanum* Noack. In the isolate from twigs, the fruit bodies are somewhat smaller than those found on the leaf and berries and the spores are a little shorter.

The death of the twigs generally begins at the tip and spreads backwards to a limited extent only; in some cases it may start in the middle and spread both ways. In full grown bushes, it commences on the primary branches here and there indiscriminately throughout the bush killing all the secondary twigs on each attacked branch except those towards the base. The affected bushes, after the disease has advanced considerably, present a mass of dead twigs on the crown region. We have not come across any instances where this disease has killed the entire mature bushes.

In many instances the attacked branches are covered with corky bark higher up and more irregularly than usual. Often a compact, black mass of filaments of a blue-green alga (*Oscillatoria*) is seen on the branches. New shoots are put out from the axils to replace the lost foliage. These bear small leaves which are often pale, crinkled and of all odd shapes, suggesting deficiency symptoms.

In Mysore, the usual die-back on coffee occurs at two main periods, one towards the end of monsoon which is mostly due to Black rot (*Koleroga knoxia* Donk) and or the leaf disease and the other after the crop in hot weather. On the other hand, the die-back associated with the new malady has been repeatedly observed after the blossom (spring) showers. The influence of physiological strain on the susceptibility of the bush to the malady should not be overlooked in this context. The strain on the bush may be due to several causes, *viz.*, entomological, pathological, edaphic and cultural.

No differences in regard to starch content of roots and shoots were found which might explain why some defoliated shoots died back whereas others recovered. Statistically designed experiments should be laid out to investigate critically whether prevention of defoliation checked die-back. *Colletotrichum coffeanum* Noack was found to sporulate heavily, especially on dead twigs with the first showers. In several instances it was seen fruiting on newly developed bark areas and twigs and a pre-blossom copper spray was found beneficial.

Germinated conidia may be found on any part of the coffee bush during the monsoon period. Probably under appropriate conditions of climate the fungus becomes parasitic causing death of the twigs. The presence of fungus mostly in the nodal region indicates that the site of attack may be the petiolar base at the point of abscission, or possibly the leaf scar is the tissue which is colonised by the fungus as a necrotroph.

It may be recalled that as a result of its wide geographical distribution it was suggested by Rayner and Jones that *Colletotrichum coffeanum* may be responsible for the premature leaf fall which afflicts East African *arabica* coffee. Just like the present die-back in Mysore the premature leaf fall has not been reproduced under laboratory conditions but the hypothesis that both these conditions are incited by a pathogenic agent is strongly supported by evidence from beneficial spraying results with copper fungicides.

In the absence of any other known organism as ubiquitous as *Colletotrichum coffeanum* this fungus remains the most likely pathogen responsible for the immediate cause of die-back associated with the new Malady of arabica coffee. By far, the most important feature of *Colletotrichum coffeanum* complex is that there may be one or more physiological strains (biotypes) which may be able to attack the host as primary pathogen(s) others requiring predisposing causes for infection.

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