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VASCULAR STREAK DIEBACK OF COCOA IN WEST MALAYSIA

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VASCULAR STREAK DIEBACK OF COCOA IN WEST MALAYSIA

by

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ABSTRACT

A detailed study into vascular streak dieback (VSD), the most serious pathological problem of cocoa in Malaysia was carried out. The causal pathogen was isolated and the symptomatology relating to infection of mature cocoa and seedling in the nurseries has been described. Morphological studies of the local isolates have confirmed the close similarity of the VSD fungus to Oncobasidium theobromae Talbot and Keane, described as the primary causal agent of VSD in Papua New Guinea. Techniques useful for isolation of the pathogen in routine and sequential samplings were investigated. The use of nutrient poor medium, surface treatment of infected materials and plating samples within 48 hours after collection from the field had improved the efficiency of isolation of the VSD pathogen. The leaf isolation technique developed is a rapid and non-destructive method for isolation of the pathogen and confirmation of VSD incidence especially in seedlings in the nursery.

A survey of the disease undertaken confirmed incidence of VSD in mature plantings in the states of Trengganu, Johor, Perak and Malacca; cocoa nurseries in Perak and Johor; and clonal plantings in Negeri Sembilan. In all cases, the percentage incidence and severity varied from locality to locality. Generally, the survey of clonal plantings had provided information on the relative susceptibility within clones.

The fungus hitherto recorded to grow poorly on artificial media, had been found to grow satisfactorily on Corticium culture medium and on Leonian's solution agar.

Sporulation of the fungus had not been previously reported in Malaysia. A method has been developed to induce sporulation under controlled environmental conditions mainly saturated humid conditions in a misting unit. Observations of sporophores had enabled comparison of local isolates with those described from Papua New Guinea which has further confirmed the close similarity of the local fungus to Oncobasidium theobromae Talbot and Keane with regards to the morphology of the sporulating phase.

Epidemiological investigations had shown significant correlation between disease incidence and rainfall. In mature cocoa pruning at regular intervals of three weeks was ineffective in reducing new infections. Disease incidence in seedlings showed gradual increase initially followed by a more rapid rise two months after observation of initial infections.

In an attempt to study the effects of systemic fungicides for controlling VSD in an exploratory trial in the nursery, significant inhibitory effects on growth of the pathogen was showed by biloxazol at 750 ppm a.i. The fungicide was subsequently tested in a larger nursery trial where significant reduction in percentage infection was obtained with biloxazol at 1500 ppm a.i. compared to control.

ABSTRAK

Satu kajian mendalam mengenai vascular streak dieback (VSD) yang merupakan masaalah penyakit koko yang paling serius di Malaysia telah dijalankan dan tanda-tanda penyakit yang berhubung dengan perjangkitan koko yang matang dan anak benih di tempat penyemaian dihuraikan. Kajian morfologi asingan tempatan telah mengesahkan rupa yang hampir sama dengan kulat VSD, Oncobasidium theobromae Talbot dan Keane yang dihuraikan sebagai agen utama yang menyebabkan VSD di Papua New Guinea. Cara-cara pengasingan patogen untuk kegunaan pensampelan lazim dan berturutan telah dikaji. Kegunaan medium yang mengandungi zat yang kurang, rawatan bahagian luar bahan-bahan yang berpenyakit dan membuat pengasingan dalam masa 48 jam setelah dipungut dari ladang telah memperbaikki kecekapan pengasingan patogen VSD. Teknik pengasingan daun yang diperkembangkan adalah cara yang cepat dan tidak merosakkan di dalam pengasingan patogen dan pengesahan kejadian VSD terutamanya di tempat penyemaian.

Satu tinjauan tentang penyakit ini mengesahkan kejadian VSD pada koko yang matang di negeri Trengganu, Johor, Perak dan Melaka; tempat-tempat penyemaian di Perak dan Johor; dan tanaman-tanaman klon di Negeri Sembilan. Kesemuanya menunjukkan bahawa peratus kejadian dan kehebatan penyakit berubah dari satu kawasan ke kawasan yang lain. Pada amnya, tinjauan tanaman-tanaman klon telah memberi maklumat tentang kepekaan relatif antara klon-klon.

Kulat yang sehingga ini dicatatkan mempunyai pertumbuhan yang kurang baik di atas media tiruan telah tumbuh dengan memuaskan di atas media pertumbuhan Corticium dan agar larutan Leonian.

Pembentukan spora kulat ini belum pernah dilaporkan di Malaysia. Satu cara telah diperkembangkan untuk mengalakkan pembentukan spora di dalam keadaan sekitaran yang terkawal ia itu melalui keadaan kelembapan yang tepu di dalam sebuah unit misting. Sporofor yang dilihat ini membolehkan perbandingan asingan tempatan dengan kulat yang dihuraikan dari Papua New Guinea dan seterusnya mengesahkan rupa yang hampir sama di antara kulat tempatan dengan Oncobasidium theobromae Talbot dan Keane dari segi morfologi diperingkat pembentukan sporofor.

Kajian-kajian epidemiologi telah menunjukkan korelasi yang bererti diantara kejadian penyakit dan hujan. Cantasan pada pokok koko yang matang pada selang masa tiga minggu tidak berkesan bagi mengurangkan perjangkitan yang baru. Kejadian penyakit pada anak benih menunjukkan pertambahan yang gradual pada peringkat awal diikuti oleh kenaikan yang cepat dua bulan setelah jangkitan pertama dilihat.

Didalam percubaan untuk mengkaji kesan fungisida sistemik bagi pengawalan VSD didalam satu percubaan exploratory di tempat penyemaian, kesan halangan pertumbuhan patogen yang bererti telah ditunjukkan oleh biloxazol pada 750 ppm ramuan aktif. Fungisida ini kemudianya diuji didalam percubaan yang lebih besar di tempat penyemaian dimana peratus perjangkitan penyakit telah dikurangkan dengan bererti oleh biloxazol pada 1500 ppm ramuan aktif berbanding dengan kontrol.

CHAPTER I

INTRODUCTION

Cocoa dieback has been associated with cocoa growing countries all over the world. It is a disorder of cocoa which is characterised by a progressive desiccation of branches beginning at the tips and gradually spreading downwards.

In Malaysia the cocoa dieback problem was first encountered in the 1950's when cocoa was introduced on a plantation scale in the country. The Amelonado cocoa planted at that time was severely affected by branch dieback. Later, it was considered to be a major factor limiting cocoa yield in Malaya and recommendation for planting was withdrawn (Haddon, 1961).

In 1971, Keane and Turner revealed the presence of a particular form of dieback of cocoa in West Malaysia. It was described to be similar to 'vascular streak dieback' (VSD) caused by Oncobasidium theobromae recorded from Papua New Guinea (Keane et al, 1972).

Since then, many authors had reported the importance of VSD in Malaysia. It was regarded to be potentially the most destructive disease of cocoa in Malaysia by Byrne (1976). With the increase in the hectarage under cocoa in Malaysia, cocoa growers were cautioned with important diseases associated with the crop and VSD was considered as the most serious in Malaysia (Ng, 1977).

According to Turner and Shepherd (1978) the dieback problem encountered in Malaysia in the 1950's which prevented establishment of a major cocoa industry was almost without doubt due to infection by O. theobromae; the truth in their statement however is open to question as no one had isolated the causal fungus and no conclusive evidence was available on the existence of O. theobromae in Malaysia.

Dieback was suspected to be caused by a multitude of causal factors including a number of fungal organisms. Following the elaboration of vascular streak dieback in Papua New Guinea (Keane et al, 1972) although the occurrence of the disease had been reported in Malaysia, research into the disease has been generally lacking here. No attempt had been made to study local isolates of VSD pathogen and to establish its identity with O. theobromae Talbot and Keane. There has also been no previous study on the general aspects of aetiology, epidemiology and control of VSD pathogen. Hence, the present study was initiated with the following objectives:

1. To isolate and identify the causal agent of VSD in West Malaysia and to compare its morphology with O. theobromae described from Papua New Guinea (Talbot and Keane, 1971).
2. To survey the incidence of VSD in West Malaysia.
3. To elucidate aspects of the biology including aetiology, epidemiology and control of VSD pathogen in West Malaysia.

CHAPTER II

REVIEW OF LITERATURE

Dieback disease of cocoa

In a detailed review of cocoa dieback, Turner (1967) recorded the occurrence to be widespread in 37 cocoa growing countries in Asia, Africa, the Caribbean, Central America and South America. Investigators in these countries attributed the dieback to one or a combination of factors which included environmental, nutritional, tree age and vigour, varietal response, pests and diseases. The symptoms and factors associated with cocoa dieback in Malaysia were also described by Turner (1968).

The number of fungi associated with cocoa dieback is colossal. More than 80 species of fungi were listed of which only two Botryodiplodia theobromae and Calonectria rigidiuscula (often in the imperfect state, Fusarium decemcellulare) were consistently isolated and considered as pathogens or potential pathogens (Turner, 1967). Botryodiplodia dieback was considered to be caused by invasion of weakened or wounded branches by the fungus B. theobromae in many cocoa growing countries including Papua New Guinea (Shaw, 1962). In Malaysia, B. theobromae, Phomopsis sp. and C. rigidiuscula were isolated from cocoa dieback tissues (Turner, 1968).

Many of these fungi have been tested for their pathogenicity by various investigators. Inoculation tests with B. theobromae and Fusarium spp. were unsuccessful in Malaysia (Voelcker, 1954). Pathogenicity tests with C. rigidiuscula had been inconclusive, some recording success and others failure (Turner, 1967). In Papua New Guinea, pathogenicity tests with B. theobromae and other isolated fungi were also unsuccessful (cited by Keane, 1972). Negative results were

also obtained in a large scale pathogenicity test with hybrid cocoa seedlings and seven isolates of F. decemcellulare in Sabah (Williams and Liu, 1976).

The occurrence of vascular streak dieback

Investigations in Papua New Guinea presented clearer evidence when Talbot and Keane (1971) had described a new genus and species of fungus Oncobasidium theobromae (Ceratobasidiaceae, Tulasnellales, Basidiomycotina) from cocoa showing dieback symptoms in Papua New Guinea. This dieback was distinguished from common dieback occurring in other countries and named 'vascular streak dieback' (VSD) by Keane et al (1972). They found O. theobromae was consistently associated with the disease. Seedlings inoculated with spores of the fungus developed typical symptoms of the disease within three months. Subsequent studies on VSD in Papua New Guinea and inoculation tests on seedlings with spores of the fungus confirmed O. theobromae as the causal pathogen of VSD (Prior, 1978).

In Papua New Guinea, VSD occurs on the mainland of New Guinea and Papua and the island of New Britain (Keane, 1981). The disease was observed to be widespread in West Malaysia especially in the Amelonado cocoa planted in the East Coast (Keane and Turner, 1972). Based mainly on the close similarity of symptoms, they reported the incidence of VSD in Perak, Trengganu and Selangor. Later, Chan and Lee (1973) reported occurrence of VSD in the five main cocoa areas in West Malaysia viz. Perak, Selangor, Trengganu, Johor and Pahang.

The distribution of VSD according to the map issued by the Commonwealth Mycological Institute in 1975 was confined to Papua New Guinea and West Malaysia. However, there have been reports of VSD in other countries in recent years. Slight occurrence of VSD had been observed in North Sumatra (Turner and Shepherd, 1978). Reliable reports

of VSD was mentioned in Sabah and Philippines (Prior, 1980). However, by studying the fungus involved, Varghese (1980) and Keane (1981) confirmed the presence of VSD in Sabah. Therefore, in contrast with the distribution of other forms of dieback, VSD was confined only to regions in South East Asia (Figure 1).

Biology of VSD pathogen

The morphology of O. theobromae, the causal pathogen of VSD, has been described in detail by Talbot and Keane (1971) and Keane (1972). These subsequently provided the necessary information for identification and comparison of the causal fungus in Malaysia.

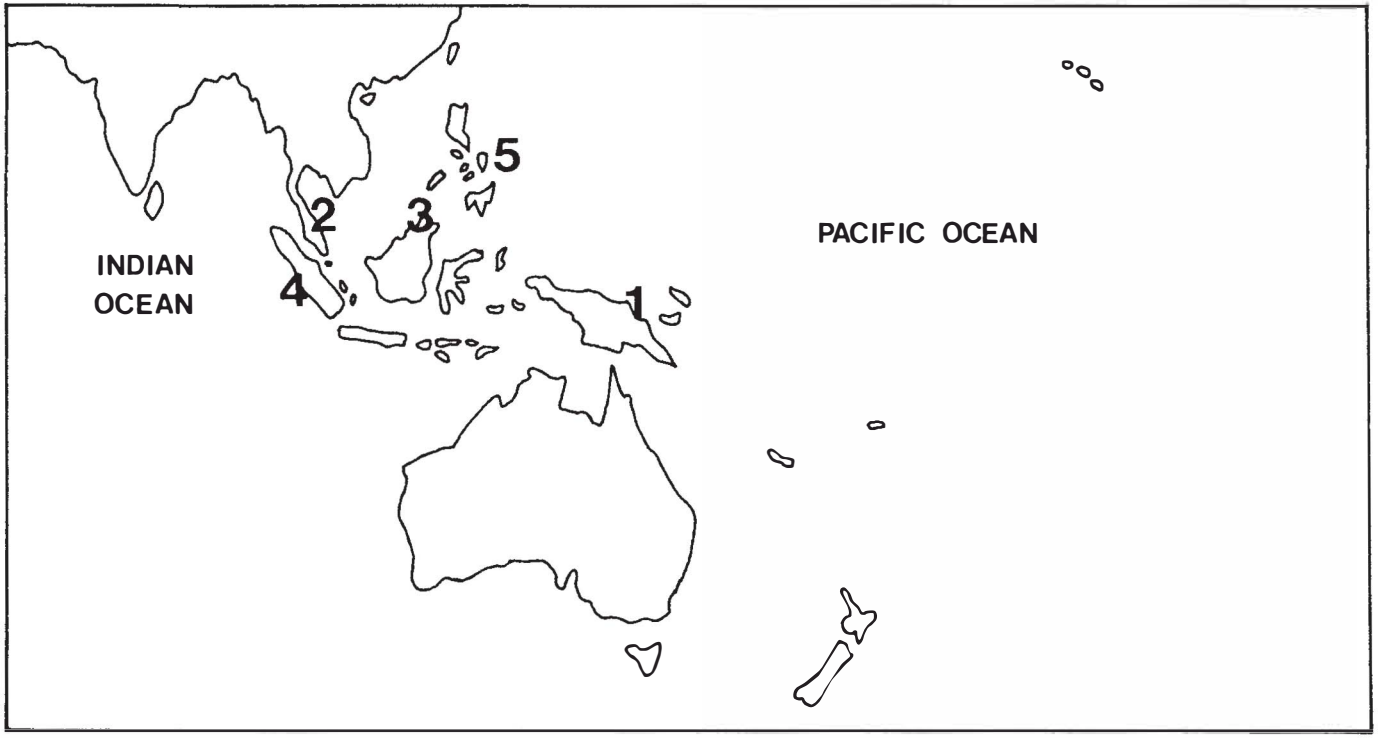
Various aspects of the biology and host-parasite relationship have been studied in Papua New Guinea by Keane (1972, 1981) and Keane et al (1972). These studies showed that basidiospores of O. theobromae penetrate young, unhardened cocoa leaves and this occurred mostly between epidermal cells overlying the leaf veins. The sub-epidermal hyphae grew along the veins thus gaining entry into xylem vessels. Sporulation of the fungus occurs on leaf scars of abscissed leaves during periods of moist weather. The authors also observed basidiospores to be shed mainly at night and are wind borne.

Sporulation of O. theobromae has not so far been observed in culture. Inoculation studies were undertaken with spores obtained from sporophores present on infected stems in the field (Keane et al, 1972; Prior, 1978). Attempts were made to establish the fungus with cocoa callus tissue to produce spores but were not successful (Prior, 1977).

Losses

The losses due to VSD especially in mature areas have been difficult to assess. According to Byrne (1976) the overall loss of production due to VSD was estimated between 25 to 40%. In Malaysia, considerable

Figure 1. Map showing countries where vascular streak dieback occurrence has been cited in literature.



Key:-

1. Papua New Guinea
2. West Malaysia
3. East Malaysia
4. Indonesia
5. Philippines

reduction in yields in new plantings occurred in areas severely affected by VSD (Shepherd et al, 1977). Deaths due to VSD in seedlings in the nursery and in immature field plantings have been frequently observed in Malaysia (Zainal Abidin et al, 1981). In Papua New Guinea while the disease was damaging in seedlings up to about 18 months old, only the most susceptible mature trees were killed (Keane, 1981). There were reports of the decline in yield of cocoa after the twelfth year of planting due to VSD, in unpruned stands in Papua New Guinea (Keane, 1981).

Control of VSD

Good cultural practices had been stressed for the control of die-back since physiological and environmental conditions had been thought to predispose cocoa to the disease. However, after being established that VSD is caused by O. theobromae pruning of diseased branches has been widely recommended and practised as a means of controlling the disease in the field. Pruning methods vary and have met with mixed success (Byrne, 1976). In Malaysia, although a pruning interval of not longer than 3 months had been suggested (Chan and Syed, 1976), evidence presented subsequently showed monthly rounds to be more effective for reducing incidence of VSD (Jayawardena et al, 1978). Regular monthly pruning was also recommended in Papua New Guinea (Prior, 1980).

The occurrence and distribution of VSD in Papua New Guinea showed that although it is common in some regions of the country and islands, it is not recorded in some of the other islands. Thus quarantine measures had been recommended as a method of control by enforcing a total ban in the movement of materials from disease infected areas to disease free areas (Prior, 1980). In Malaysia, it was suggested that movement of cocoa vegetative planting material from VSD areas to

VSD free areas should be banned (Byrne, 1976).

Chemical control of VSD has been difficult mainly due to the fungus responsible being active within the vascular tissue of the host. The possibility of using systemic fungicides had been suggested (Keane and Turner, 1972; Chan and Syed, 1976; Prior, 1980); although none had so far been recommended.

Selection of resistant planting materials is perhaps the most practical approach to long term control of VSD. The decline in the severity of VSD which occurred in epidemic proportions in the early 1960's in Papua New Guinea had been attributed to be partly due to the distribution of resistant clonal materials (Prior, 1978; 1979). Screening of resistance in cocoa seedlings and clonal cuttings had been attempted by inoculating spore suspensions of O. theobromae in the upper surface of a young unexpanded leaf and onto the stipules of the apical bud (Prior, 1978). Although considerable variation in resistance and susceptibility had been observed by this method, no cultivars had been observed to be completely resistant to VSD. In Malaysia according to field observations of natural infections, Amelonado varieties were observed to be very susceptible compared to Upper Amazon varieties which were apparently more resistant (Haddon, 1961; Turner, 1968; Keane and Turner, 1972; Chan and Lee, 1973; Chan and Syed, 1976). Hybrid progenies of Sca 6, Sca 12 and Na 33 have been found to possess significant tolerance or resistance to VSD in Malaysia (Ang and Shepherd, 1978; Turner and Shepherd, 1978).

CHAPTER III

SYMPTOMATOLOGY

The symptoms of vascular streak dieback of cocoa in West Malaysia were observed to be similar to those recorded in Papua New Guinea (Keane and Turner, 1972). In the present study attention was given to describe the symptomatology in detail mainly as an aid to correct diagnosis of the disease in the field and also in cocoa nurseries in Malaysia. In view of this regular observations were made on the symptoms of the disease where involvement of VSD pathogen was confirmed by isolation. The symptomatology of the disease on mature cocoa and immature cocoa including seedlings are described in detail below:

Symptoms on mature cocoa

An early sign of the disease is the appearance of partial or total paling of the lamina of leaves in a specific flush (Figure 2). As the symptoms developed further, chlorosis accompanied by dark green mottlings become evident. In the later stage, the chlorosis extends over the whole leaf. The size of mottles decreases and abcission normally occurs prior to unaffected leaves or leaves which are not in the advanced stage of infection on the same branch.

The sequence of development of symptoms in mature leaves is shown in Figure 3. These symptoms are usually observed in the second or third flush leaves behind the growing point (Figure 4). However, these symptoms may also be observed in hardened terminal flush leaves (Figure 5).

An accompanying secondary symptom is the streaking within the vascular tissue of infected branches, which is useful in diagnosing VSD infections in the field.

Figure 2. Early leaf symptoms of VSD.

Figure 3. Sequence of development of VSD symptoms in leaves.
a - healthy b, c - partial paling
d - chlorosis and green mottling

Figure 2

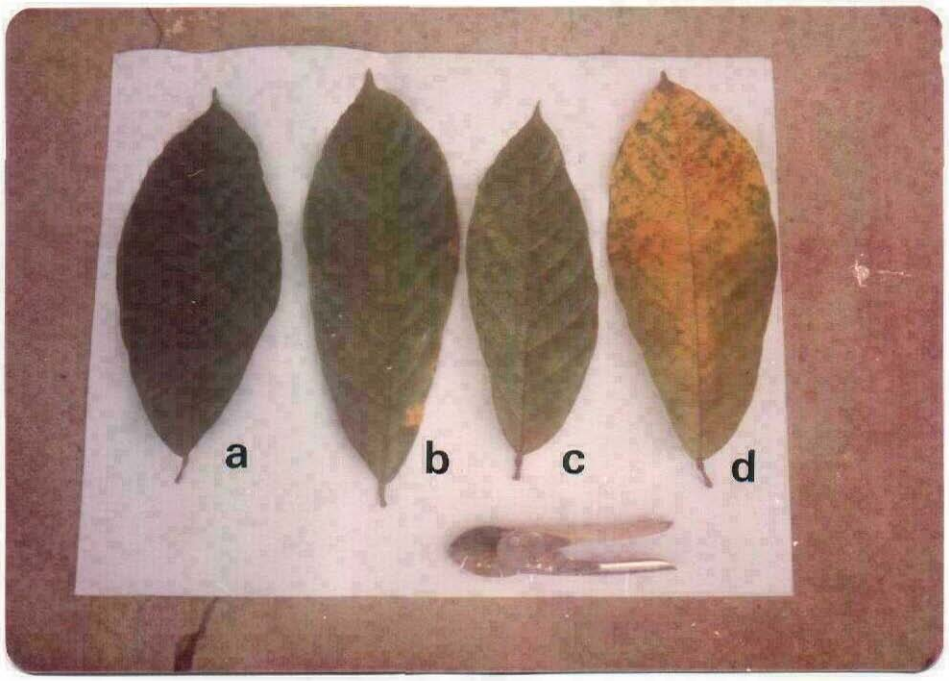


Figure 3