A Fusarium Wilt (*Fusarium oxysporum*) of Angsana (*Pterocarpus indicus*) in Singapore.

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Abstract

The Angsana wilt disease affecting *Pterocarpus indicus* in Singapore and caused by *Fusarium oxysporum* was first reported in Malacca in 1870. Following several outbreaks in various parts of the Peninsular Malaysia the disease was recorded in Singapore in 1914 and by 1919 many of Singapore's Angsanas had either been killed by the disease or removed to prevent its further spread. Sporadic occurrences of the disease occurred around Singapore between 1970 and 1982 resulting in a rapid investigation of the disease and the implementation of control measures. Between 1980 and 1992, more than 800 Angsanas were removed as a consequence of the disease.

Although both *F. oxysporum*, and *F. solani* were consistently isolated from infected trees only *F. oxysporum* proved to be pathogenic in inoculation experiments.

During a 10 month period, 170 Angsana trees were inspected because they had symptoms similar to the Angsana wilt disease. Of the 170 trees, 86% (147) were infected with *F. oxysporum*, while the other 14% were the result of lightning strikes. Of the 147 infected trees, 90% had also been struck by lightning and 87% had both lightning and ambrosia beetle infestations. The remaining 15 trees (10%) which were not struck by lightning were at secondary infection sites where an adjacent Angsana had already been removed because it was infected with *F. oxysporum*.

The hypothesis presented here for the life cycle of the Angsana wilt disease is that lightning damage to an Angsana, provides the stress which attracts the ambrosia beetles. If these beetles are contaminated with *F. oxysporum* spores, then infection is likely to follow. The secondary spread away from this primary infection site, is by *F. oxysporum* which has entered the soil from the infected tree.

Short term control strategies are discussed which include the rapid removal of all lightning damaged trees and the use of insecticides and fungicides either sprayed or injected to prevent the establishment of new infection sites.

Long term control is anticipated following screening of Angsanas collected from a wide geographical area, and selection for resistance to *F. oxysporum*.

Key Words: Ambrosia beetles; Angsana; Angsana wilt; *Fusarium oxysporum*; *Fusarium* wilt; injection; lightning; *Platypus parallelus*; *Pterocarpus indicus*; resistance.

1 INTRODUCTION

The wilt disease of Angsana (*Pterocarpus indicus*) caused by *Fusarium oxysporum* was responsible for killing on average about 28 trees a month in Singapore between 1989 and 1995. The first indication of infection is the yellowing of leaves on one branch followed by their death. This is followed by the yellowing of leaves on subsequent branches until the tree is completely killed (**Plates 1.1, 1.2**), a process which can take either a few weeks or many months. Infection is confirmed by the presence of darkened xylem vessels within the primary xylem and the isolation of *F. oxysporum*.

Plate 1.1
An Angsana infected with the Angsana wilt disease. The disease has already killed one of the branches, and a second branch is starting to show the early signs of infection. The leaves turn bright yellow before turning brown and falling. This magnificent tree was the last in a avenue of about 10 trees.

Plate 1.2
The same tree five weeks later showing the rapid progress of the disease.

The disease was first reported in Malacca, on the South-west coast of Malaysia, where between 1870-1880 the disease practically wiped out a magnificent avenue of trees which adorned the sea shore (Fox, 1910).

Thirty years later the disease started to appear in other areas of Malaysia, where, between 1906-1910, around 100 trees were killed by the disease on the island of Penang and at about the same time some Angsanas in Tapah (Perak) were also killed. Soon after, many trees died in Kuala Kubu , Kuala Lumpur (Selangor) and in Taiping (Perak) (Bancroft, 1912) .

The disease first appeared on Pulau Brani, an island in the port of Singapore, during 1914. From there it jumped to Connaught Drive on the waterfront, then a kilometre inland to Dhoby Ghaut. It subsequently appeared in the grounds of the Istana where the disease developed in an avenue of Angsanas near the gate to Orchard Road. The avenue was immediately cut in the hope of restricting the spread of the disease (Burkill, 1918). A localised strain of this disease is still endemic in these grounds today (Crowhurst et. al., 1995).

The disease continued inland during early 1919 and by May some trees at the end of an avenue at Tanglin Barracks, 5 kilometres inland showed sign of Angsana wilt. Although the disease spread to many other parts of Singapore, the epidemic was held in check by the rapid removal of any tree showing signs of the disease (Furtado, 1935b).

Sporadic occurrences of the Angsana wilt disease occurred around Singapore between the late 1970s and 1982, however, an outbreak which affected 28 trees in an avenue of Angsanas along Tampines Road, resulted in a rapid investigation into the disease. This investigation culminated in a report written by John Harden (1982) who formulated a number of control measures which were implemented. These included the removal of trees once they were confirmed to be infected, with the trunks and branches being burnt. The areas around infected trees, and around trees suspected of being infected, were drenched with fungicides. Field officers were advised to closely monitor any further spread of the disease and strict horticultural sanitation was introduced.

With the removal of the infected trees and the intensive soil drenching with fungicides, only 8 further infections were encountered during the next year and the spread of the disease again appeared to be under control. The next report of trees being removed because of Angsana wilt was in October 1988, when two Angsanas along Collyer Quay were found to be diseased. Since 1988 this latest epidemic has gathered momentum and over 800 trees have been removed as a consequence of the disease, by the Parks and Recreation Department's Maintenance Division.

During 1990, the Parks and Recreation Department, because of their concern

regarding this disease, organised a seminar and a field trip to familiarise field officers with this disease. Later in that year a joint application was made by National Parks Board, Primary Production Department and the Parks and Recreation Department to the National Science and Technology Board and to SGS SINGAPORE Pte. Ltd, for funding to study this disease.

2 THE CAUSAL ORGANISM

During the 1982 investigation, the causal organism was tentatively identified by Professor Gloria Lim and Fong Yok King, and confirmed by the Commonwealth Mycological Institute, as *Fusarium oxysporum*. Similar wilt diseases occur on *Pterocarpus angloensis* in Africa (Piearce, 1979), and on *Albizzia julibrissin* (Pirone, 1988) in the United States. No successful pathogenicity test was conducted with the fungus at that time.

Fusarium oxysporum is a common pathogen of crop plants causing considerable economic losses in peas, beans, tomatoes, cotton and bananas. There are also examples of *F. oxysporum* being pathogenic to palms where it is of commercial importance on both oil and date palms (Turner, 1981; Louvet and Toutain, 1973). It has also been recorded on ornamental palms in Singapore on a number of occasions by staff of the Plant Health Diagnostic Branch of the Primary Production Department in Singapore (PPD Disease Records).

2. 1 Isolation

During the preliminary investigation a number of Angsanas showing symptoms of Angsana wilt were sampled and fusaria isolated. The isolates consisted of what were considered typical *Fusarium oxysporum* with short phialides and typical *Fusarium solani* with long branched phialides (Toussoun and Nelson, 1968; Booth, 1977; and Burgess *et. al.*, 1988). There was also, however, a wide range of fusaria covering the entire range from short *oxysporum*-like, to long *solani*-like phialides.

During November and December 1991 thirteen sites consisting of 61 trees showing symptoms of Angsana wilt were sampled. The dead areas of the bark were identified by removing the outer bark (**Plates 2.1, 2.2, 2.3**) using a sharp, 3 cm wood chisel and hammer. The inner bark, together with a thin portion of underlying wood was removed and taken as the sample. Between 3 and 5 samples were taken per tree. The first sample was taken at the vertical boundary between healthy and diseased tissue, with subsequent sampling across the dead tissue to the opposite boundary. The samples were placed in a plastic bag which was then labelled.

Plate 2.1
Chopping back the bark reveals the demarcation between the healthy beige tissue and the brown to very dark brown *F. solani* infected dead bark. Between the bark and the wood are many pockets of *F. oxysporum*. The vascular staining of the *F. oxysporum* infection can be seen in the small area of exposed wood at the bottom of the plate. The holes made by the ambrosia beetles are frequently to be found associated with the infected tissues.

In the laboratory, the samples were first washed in clean tap water and then for 2 minutes in a 20% sodium hypochlorite solution (1:5 Chlorox® and water), rinsed in sterile distilled water to remove any excess chlorine then transferred to a 15 cm glass petri dish containing sterilised moist filter paper, for incubation. The samples were checked daily for developing fungal growth and spores were transferred to PDA. (Potato Dextrose Agar).

From the 61 trees sampled, *Fusarium oxysporum* was isolated from 56, and *Fusarium solani* from 45. From 40 of the 56 (71%) trees from which *F. oxysporum* was isolated, *F. solani* was also isolated, suggesting that this is a disease complex involving both fusaria.

For all samples collected later in the project, the inner bark and a thin layer of the outer wood was removed and discarded. This exposed the primary xylem, which if infected with *F. oxysporum*, would show the characteristic vascular staining (**Plates 2.4**). Where possible samples were collected from areas invaded by the ambrosia beetles. Such samples of primary xylem usually resulted in pure cultures of *F. oxysporum* (**Plate 2.5, 2.6**).

Plate 2.2
A small Angsana transplated into contaminated soil has become infected with the Angsana wilt disease (a). Removing the bark from the buttress and lower trunk has exposed the discoloration of the tissues underneath. The discoloured tissues are invaded by both *F. solani* and *F. oxysporum* (b).

Plate 2.3
Infection starting from the roots and spreading upwards into the trunk is a good indication of secondary spread.

Plate 2.4
Woody tissue of a diseased Angsana showing the brown streaks of the infected xylem vessels, the presence of ambrosia beetle holes and white powdery frass.

2. 2 Identification

Both *Fusarium oxysporum* and *Fusarium solani* were tentatively identified by the project team, using the criteria of phialide characteristics, macro-spore size, colony edge morphology, and culture colour (**Table 2.1, Plates 2.7, 2.8**), as set out in the keys of Toussoun and Nelson (1968), Booth (1977), and Burgess *et. al.* (1988).

Table 2.1 Culture characteristics of *Fusarium oxysporum* and *Fusarium solani*

	F. oxysporum	*F. solani*
Culture pigmentation		
Top	white and fluffy	white range of colours from light brown through yellow to orange.
Bottom	turn peach to salmon pink	amber to red turning dark brown or black.
Phialide characteristics	short and simple	long and branch
Macro-conidia size	20 – 32u × 3 – 5u	40 – 75u × 4.5 – 7u
Colony edge morphology	irregular with micro-conidia produced within the agar	smooth uniform hyphae

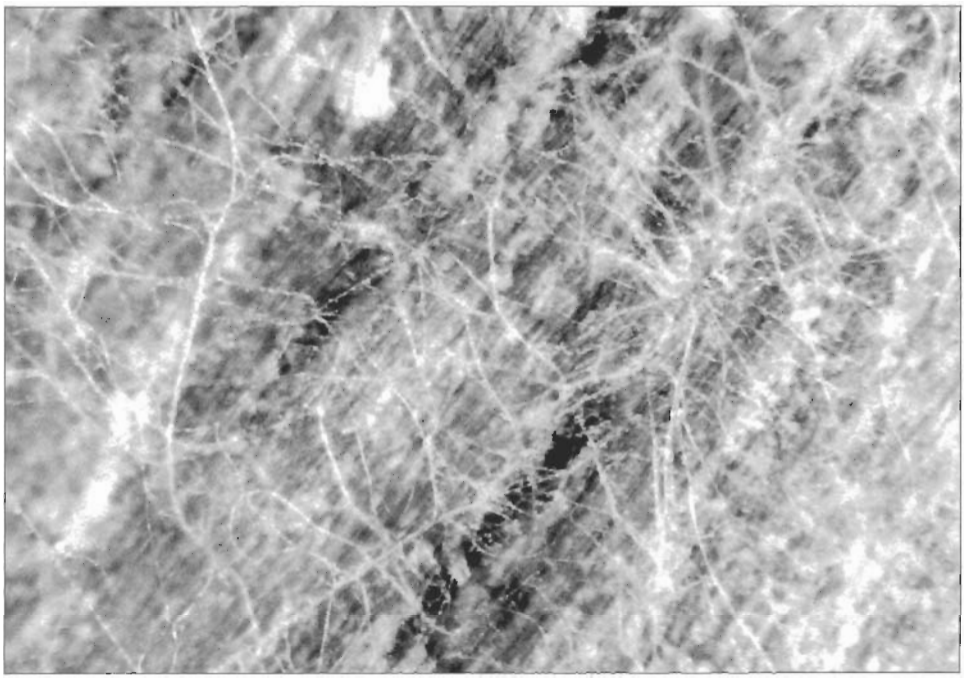


Plate 2.5
Close up view of the same colonised vessel. Bunches of conidia of *F. oxysporum* on short phialides characteristics of this fungus, can be seen on the mycelium.

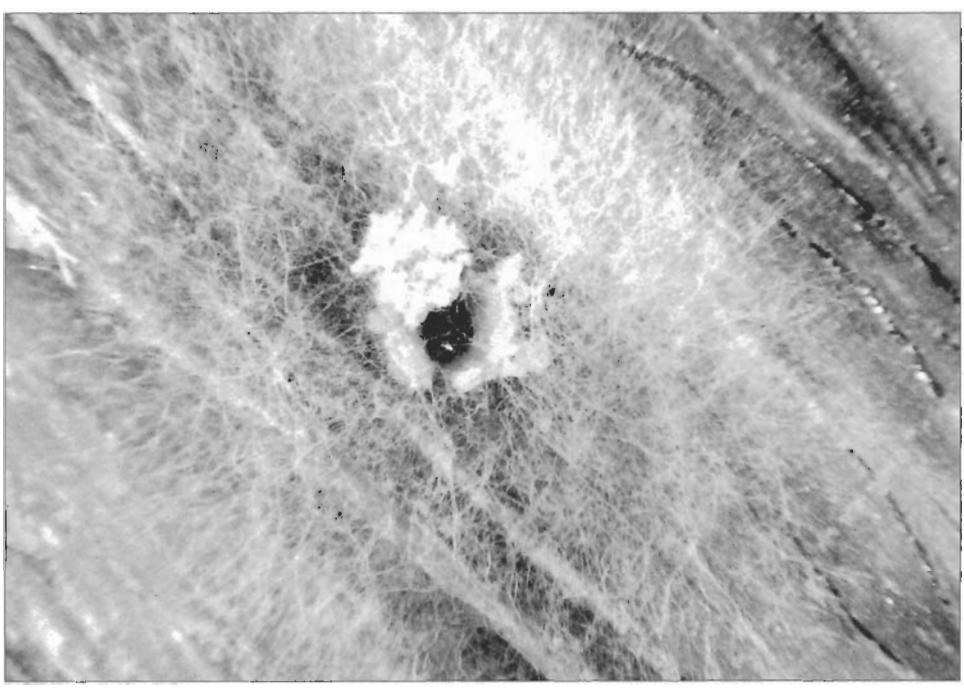


Plate 2.6
An ambrosia beetle coming out from its hole in surface sterilized diseased wood. The white cottony mycelium of *F. oxysporum* can be seen growing from the frass thrown out by the insect.

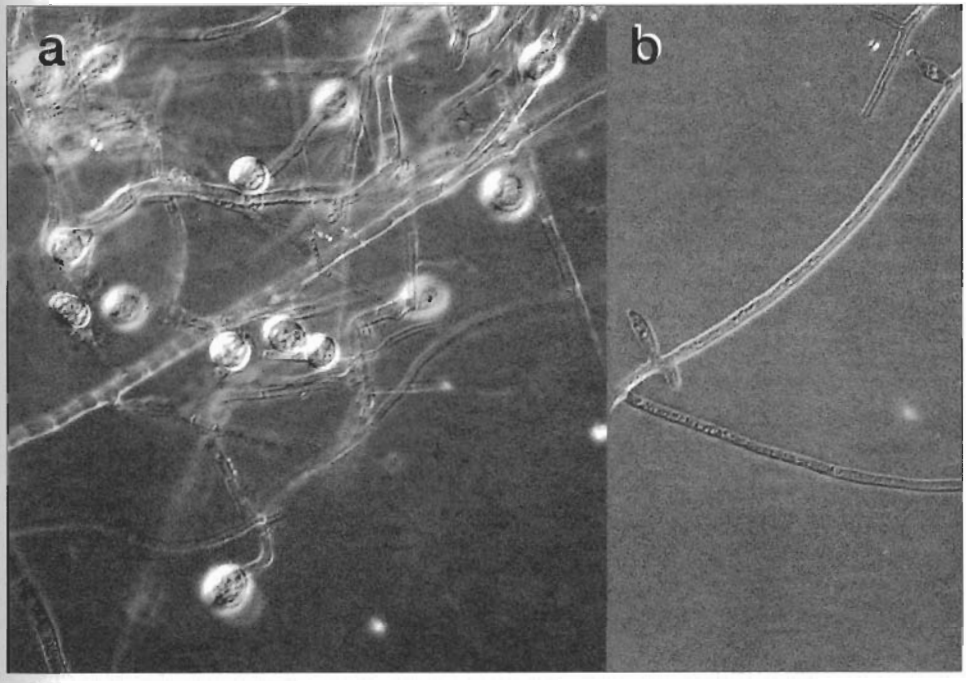


Plate 2.7
Slide made from *F. oxysporum* growing on surface-sterilised diseased wood tissue collected in Singapore. (a) Chlamydospores, (b) mycelium with short simple phialides.

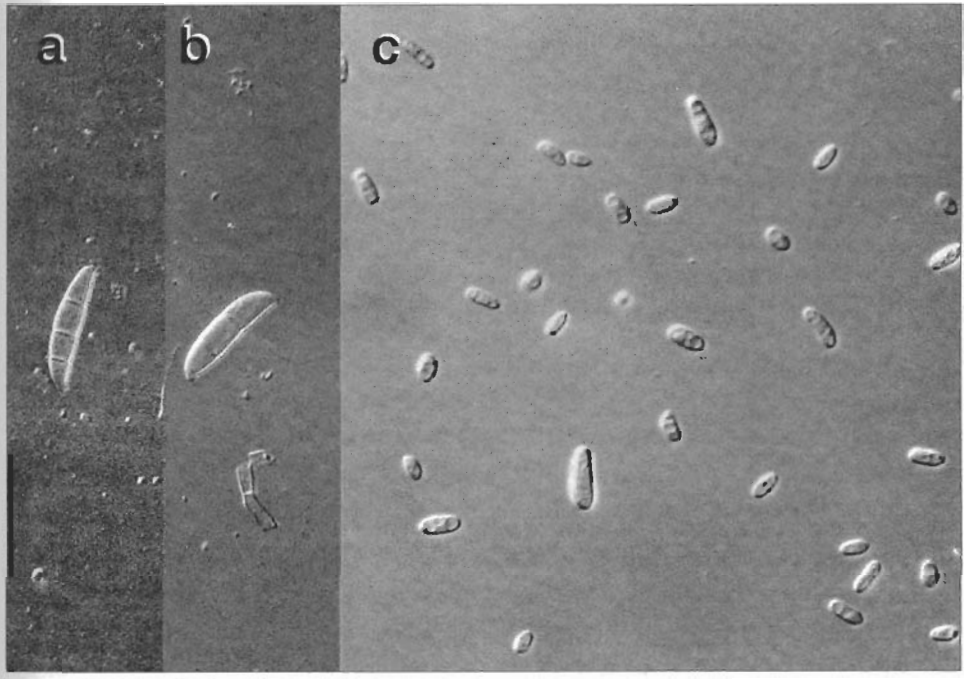


Plate 2.8
F. oxysporum isolated from diseased Angsana tissue collected in Singapore. Sizes of macro-conida range from 18-28µ (a), (b) and micro-conidia 4-14µ (c).

For confirmation of their identity, three isolates of each species were sent to the International Mycological Institute, UK, Sydney University's *Fusarium* Research Laboratory, Australia and the Crown Research Institute, Auckland, New Zealand during the course of the investigation. The three isolates of *F. oxysporum* and *F. solani* represented the range within the population. All three institutes to which the isolates of *F. oxysporum* were sent, confirmed their identity, however, with the *F. solani* isolates, there was no consensus as to their identity. In two instances different names were given to the different isolates. This is not surprising because of the variation between the *F. solani* isolates. Some isolates of *F. solani* were impossible to differentiate from *F. oxysporum* on phialide characteristics when viewed on wood, the difference only became obvious because of colony morphology when the isolates were growing on PDA. A complete range of phialide length along with a wide range of colony colour was found within the population of *F. solani*. It is suggested that when isolates were sent for identification they were seen as individuals from separate populations, rather than the natural variation within a sexually active and therefore a segregating population.

As we are not in a position to judicate on the taxonomy of the *F. solani*, and to prevent further confusion in the literature, *F. solani* is retained for this publication.

Fusarium solani was consistently isolated from the dead bark outside the *F. oxysporum* infected primary wood tissue. It was also consistently isolated from ambrosia beetles sampled. The status of *F. solani* in this disease complex, therefore warrants further investigation.

2.3 Pathogenicity Test

A root inoculation test was conducted to test the pathogenicity of the two fusaria isolated. The two fusaria were consistently isolated from the wood material collected from infected trees.

The method used was that described by Burgess et. al. (1988) in the Laboratory manual for *Fusarium* Research, and by Piearce (1979).

One hundred and fifteen, well rooted hardwood cuttings of Angsana were removed from their pots and the soil thoroughly washed from the roots. The roots were then trimmed to 50% of their original length before the cuttings were placed in a spore suspension and left in the sun for four hours. The spore suspension was made by macerating three plates of one-week old fungal lawns in 500 ml of sterile water.

Sixty cuttings were inoculated with *Fusarium oxysporum*, 45 with *F. solani* and 10 inoculated with macerated PDA as controls. After four hours the cuttings were re-potted using the original soil and watered with the fungal lawn suspension. No watering was done for the next three days, and subsequently every three days.

The first cutting started to wilt after 19 days with the majority of the cuttings wilting between days 21 and 28. The trial was terminated after 35 days.

As can be seen from the results of the inoculation test (**Table 2.2, Plate 2.9**) the only deaths occurred with those hardwood cuttings inoculated with *F. oxysporum* where 96% of the hardwood cuttings died. The identity of the causal organism was therefore confirmed as *F. oxysporum*.

Table 2.2 Pathogenicity test of the two fusaria isolated from diseased Angsanas

Angsana / Fungus	Number inoculated	Number died	Number survived	% death
F. oxysporum	60	58	2	96
F. solani	45	0	45	0
Control	10	0	10	0

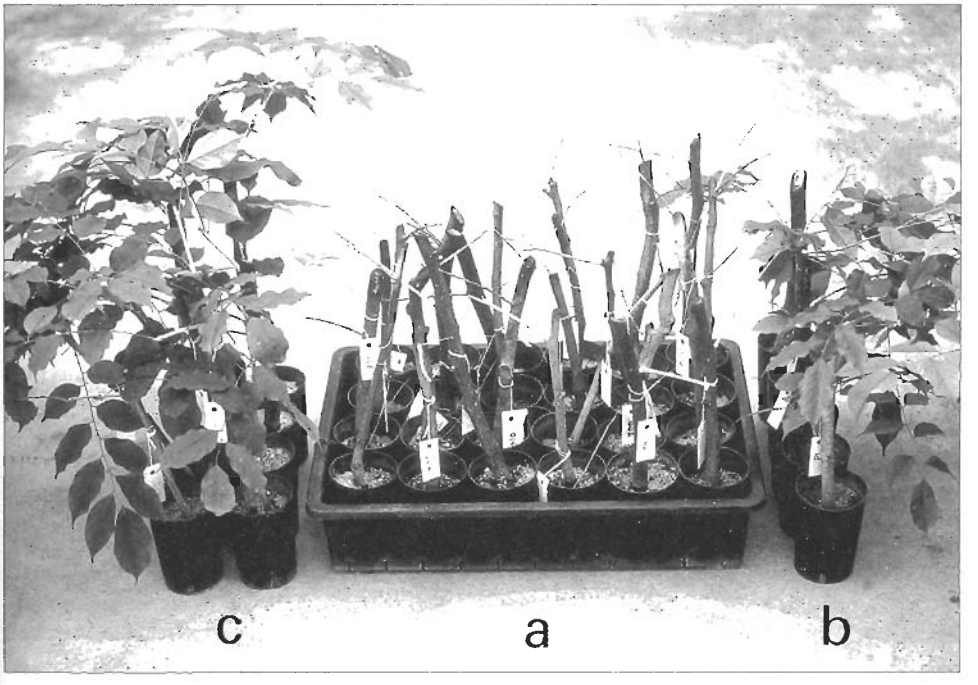


Plate 2.9
Well rooted hardwood cuttings of Angsana inoculated with *Fusarium oxysporum* and *Fusarium solani*. Five weeks after inoculation, all except 3 cuttings inoculated with *F. oxysporum* showed symptoms of Angsana Wilt Disease and died eventually (a). Those cuttings which were inoculated with *F. solani* (b) and those uninoculated plants (control) (c) remained healthy.

3 EPIDEMIOLOGY

3.1 Lightning

Damage to trees caused by lightning has been the subject of periodic publications in Singapore (Thomas, 1900; Fox, 1910; Futardo, 1935a). Osmaston

(1920: page 120) made the statement *"From general observations which are not, however, based on definite countings, I believe that under existing conditions (in Asia) about 50% of trees struck (by lightning) survive, and the death of the remaining 50% is, I believe, mainly brought about by insect and perhaps also fungal attack. On more than one occasion I have noticed how Platypus biformis and bark beetles may at once attack a struck tree commencing at first on either side of the rift in the bark and thence gradually extend their operations completely round the stem."* Sharples (1933) suggested, that in the case of rubber trees (*Hevea brasiliensis*), lightning discharges rendered these trees susceptible to the attack of parasitic insects or fungi.

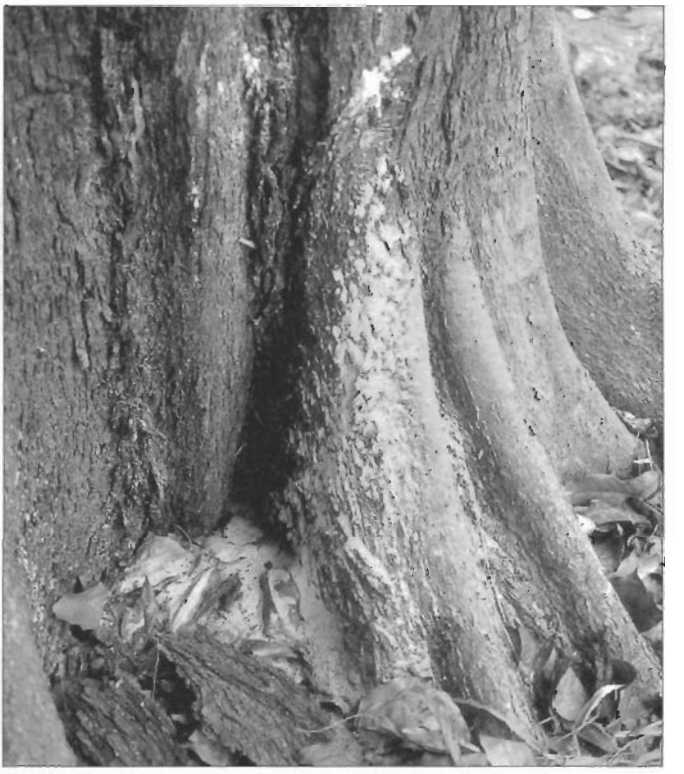
The relationship between lightning and insect attack has received considerable attention (Miller and Keen, 1969; McMullan and Atkins, 1962; Anderson and Anderson, 1968; Anderson and Hoffard, 1978 and Schmitz and Taylor, 1969). Taylor (1974 : page 843) comments that *"several genera of forest insects apparently respond to the olfactory attraction of volatile extractives released by a tree newly ruptured by lightning, and the few initially attacking insects may create sexual stimuli that trigger a mass attack on the struck tree and its neighbours."*

Harden (1982) in his report to the Parks and Recreation Department, suggested the possible involvement of the ambrosia beetles in the disease cycle of the Angsana wilt disease. However, such a suggestion posed the enigma that ambrosia beetles only infest weakened trees. For this reason it was generally assumed that the ambrosia beetles infested the Angsanas only after they had been weakened by Angsana wilt.

The puzzle which confronted the project team at the beginning of their investigation was, if *F. oxysporum* is a soil borne organism, how do new infection sites develop several kilometres away from an old infection site, and are the ambrosia beetles, as suggested by Harden, and which are nearly always associated with the infected trees, part of the disease process.

The Housing Development Board's tree nursery along University Road, gave us our first indication that lightning might be associated with the epidemiology of the disease. This occurred when a tree growing adjacent to four trees which had been unsuccessfully stem inoculated with *F. oxysporum*, attracted our attention because of a line of frass which extended from the ground to the tip of a main branch (**Plates 3.1, 3.2**). On close inspection the tree exhibited all the symptoms of Angsana wilt, and *F. oxysporum* was isolated from the tree. This situation caused considerable confusion and debate as to why an adjacent tree, and not one of the inoculated trees, should develop the symptoms of the disease. During the following weeks, however, obvious symptoms of what we perceived to be lightning damage began to appear. After three months a 5 - 10 cm strip of bark had peeled off, revealing the wood from just above the ground level to the top of the main branch.

Plate 3.1 & 3.2
White powdery frass on the Angsana trunk and root flare indicating the presence of the ambrosia beetles actively invading the tree. When this photograph was taken the leaves on the affected branches were drooping and dull green, suggesting that it had only been a few days since the lightning strike. The tree was the third in a row to have been affected.



This was the area which had originally been the site of the ambrosia beetle invasion. The disease subsequently spread to two neighbouring trees.

Of the 21 trees investigated during the following five weeks from which *F. oxysporum* was isolated, 17 of the trees also exhibited symptoms of what we suspected were caused by a lightning strike. These symptoms included, the rapid defoliation of one or several branches of the tree, areas of thin outer bark which had been lifted off in large sheets, and a line of cracked bark down the tree, defining the path of the lightning. The bark along the cracks often appeared to have been burnt or finely fragmented. Fifteen of the 17 lightning trees were also colonised by ambrosia beetles within days of the lightning strikes, with numbers of 500 - 2,000 beetles/m^2 being common.

3.1.1 Water Solubility Of Resin

Because there appeared to be an association between lightning damage and trees infected with Angsana wilt, it was important to determine with certainty, whether or not a tree had been struck by lighting. To this end we started looking at ways to confirm lightning strikes on Angsana.

An observation that the red stains on clothes, caused by the resin from Angsanas, could be washed out by vigorous scrubbing in cold water, but if the clothes were washed and subsequently ironed, then the stains became permanent and impossible to remove, led us to investigate the solubility of the Angsana resin.

It was found that the resin from both the healthy Angsana, and those colonised by *F. oxysporum* and *F. solani*, although not soluble in organic solvents, was soluble in water. Conversely, resin from the bark samples suspected of having lightning damage, were not water soluble.

To determine the temperature at which the resin became insoluble in water, 1.5 g samples of air dried, healthy bark were placed into open glass petri dishes, and heated in an oven. One petri dish sample of bark was removed at every 10°C rise in temperature between 70°C and 220°C. A glass petri dish lid was placed over the petri dish as it was removed from the oven. When these samples were placed in water, it was found that the resin was water soluble for those samples which had been removed at a temperatures of 150°C and below, whereas the resin was no longer water soluble for those samples heated to 160°C and above.

Assuming that the only natural way for the bark to be heated to temperatures above 160°C is the passage of a very high electric current through the bark, i.e. lightning. We had developed a very simple test to determine whether or not an Angsana has been struck by lightning. The test results can be obtained within five minutes, with complete repeatability, and thus a very high degree of accuracy.

3. 1. 2 Tree Survey: November 1993 - August 1994

Each week, from November 1993 until August 1994, trees showing symptoms of Angsana wilt (**Plates 1.1, 1.2**) were inspected and detailed records made of lightning symptoms, location, ambrosia beetle infestation, percentage of the canopy and bark affected, size of tree, and the number of nearby Angsana. Bark samples were collected from both the suspected lightning damage and adjacent trees. These were tested in the laboratory for the water solubility of the resin, to determine whether a tree had or had not been struck by lightning.

During the 10 month period, 170 Angsanas were inspected because they had symptoms similar to Angsana wilt. Of the 170, 147 (86%), were infected by *F. oxysporum*, the remaining 23 (14%) had been struck by lightning but no infection was detected. Of the 147 infected trees, 132 (90%) had been struck by lightning and 128 (87%) had both lightning and ambrosia beetle attack.

All the 15 (10%) trees where lightning damage was not recorded, were secondary infection sites being adjacent to a site where a tree had already been removed because of the Angsana wilt disease. The time taken for the secondary infection to move from an infected tree to the adjacent tree varied from 22 days in the case of two trees in Holland Avenue, to 131 days between two adjacent trees at the Pioneer Rd/International Rd corner. The mean time taken for the secondary infection to move between two trees was 73 days (15 observations).

3. 1. 3 Symptoms Of Lightning Damage

The first symptoms occur at the time of the lightning strike and these are the physical damages that are associated with the expansion of gases as the liquids in the bark are heated past their boiling points. In the majority of cases, sheets of outer bark, usually in the order of 50 × 20 cm, are either lifted off together with the thicker inner bark or they are completely blown off the tree (**Plate 3.4**). Small areas (1 × 5 cm) of inner bark may also be completely disrupted producing an area of loosely held fibres (**Plates 3.5, 3.6**).

During the days following the strike it becomes apparent that the tree has sustained a lightning strike as the leaves dry up, turn a dull green then brown before falling (**Plate 3.7**). If the tree is left standing, the affected branches will flush with new leaves, which may also wilt before reaching full size. In the absence of the *F. oxysporum* fungus, death or survival of a lightning struck tree depends on many factors including the size of the tree and the extent of the lightning strike.

During this period the bark along the path of the strike will start to dry and crack. It is along the edge of these cracks that the resin appears to have been baked and it is from this area that samples are collected for testing the water solubility of the resin. The drying and cracking of the bark continues over the next few

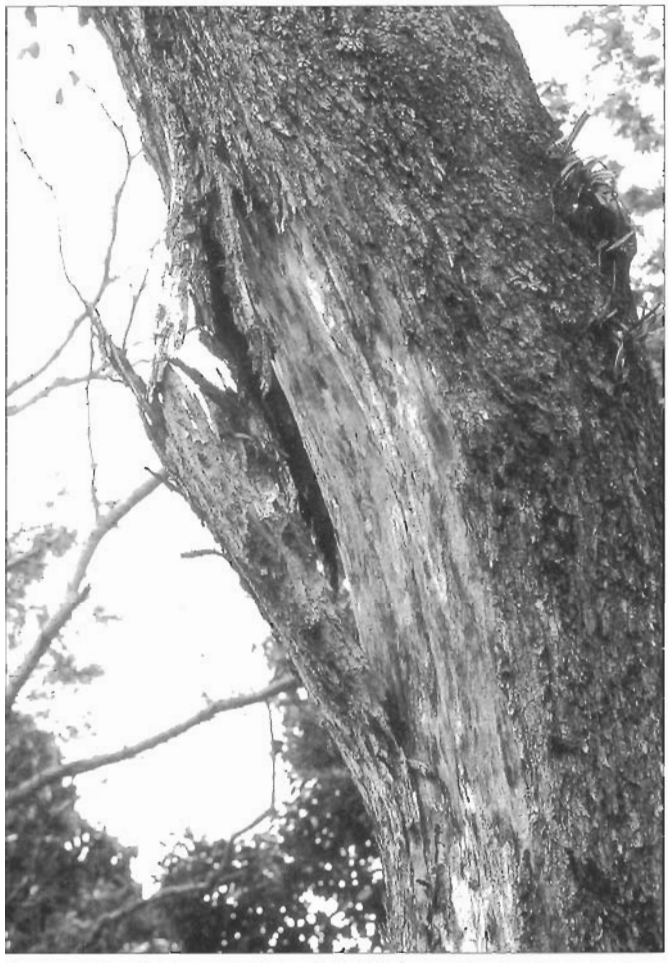


Plate 3.4
In the majority of severe lightning strikes, large sheets of outer bark get lifted off from the trunk.

months until the bark falls to the ground and the area of damage is outlined by the absence of bark (**Plate 3.8**).

It was common to record more than one tree which had apparently been struck during the same storm. In one instance a group of five neighbouring trees all showed similar symptoms (**Plate 3.9**). It is also suspected that trees which show symptoms within a few weeks of the first tree dying, might also have been struck at the same time. This delay has been attributed to the shock being transferred through the root system (Futardo, 1935a).

3. 1. 4 Ambrosia Beetles

Dutch elm disease is the best known example of a disease - insect relationships because of the widespread death of elms in both Europe and the United States. The insect-pathogen relationship is a passive one, with the fungal spores sticking

Plate 3.5
Below the sheets of thin outer bark which have been separated from the inner bark, are the vertical cracks and often small areas of completely disrupted bark producing areas of loosely held fibres.

Plate 3.6
Large sheets of outer bark are separated from the inner bark revealing the vertical cracks and areas of disrupted fibres.

Plate 3.7
An Angsana along Adam Road showing what appeared to be the symptoms of Angsana wilt disease. On close inspection the trunk showed damage due to a probable lightning strike. The leaves turned dull green then brown before falling. This process took about 7 days.

Plate 3.8
The area of exposed wood showing the old ambrosia tunnels, which three months earlier had delimited the area of damage and from which *F. oxysproum* had been isolated.

Plate 3.9
Two of a group of five Angsanas at a vacant plot of land on Adam Road, all showed similar lightning damage, suggesting that all five had been struck simultaneously.

to the insects before the adults leave the diseased tree, then being physically brushed off in the new feeding tunnels.

Ambrosia larvae on the other hand feed on fungi which are cultivated by the adults, and the spores of these fungi are actively carried from one tree to another in special pouches, mycangia, on the thorax of adults. The ambrosia beetles therefore actively inoculate newly colonised trees with their ambrosia fungi. It is not surprising therefore that when a plant pathogen becomes involved as part of this fungal-insect symbiosis that a fungal-insect-disease situation arises. Current research in New Zealand suggests that an ambrosia beetle (*Platypus spp.*) might also be involved as a vector of Dutch Elm Disease in that country (Scott C. *pers. comm.*).

In 1973 Zimmermann, found *Fusarium javanicum* to be among a range of fungi colonising the galleries produced by the ambrosia beetle, *Xylebrus*. Appreciating the significance that some of these fungi might be plant pathogens, he tested them in the laboratory and found that *F. javanicum* was indeed a pathogen, which caused a canker on tomato shoots.

The first link between an ambrosia beetle being a vector of a plant disease was when Kessler (1974) suggested that the apparent symbiosis between a *Fusarium* species and ambrosia beetle (*Xylosandrus remanus*) causes black walnut canker,

a link that was confirmed by Weber (1979, 1985). Hara and Beardlsey (1976) studied the biology of the black twig borer, a severe pest of shrubs and trees, causing extensive economic damage to coffee and cacao in tropical Africa, Indonesia and Southern India and to tea in Japan, and found that the eggs were laid on the ambrosia fungus, *F. solani*. The symptoms of the attack were necrosis of leaves and stems, which extended from the entry hole to the terminal shoots of the branch. These are leaf and stem symptoms characteristic of *Fusarium* attack on crop plants such as peas and beans.

In 1978 Anderson and Hoffard demonstrated the link between *Fusarium* canker (*F. solani*), and the ambrosia beetle *Xylosandrus germanus* and *Xylebrus sayi* on tulip poplar trees in Ohio.

There are three reported examples of ambrosia beetles being associated with diseases of pines. Frederick (1976) provided data which suggested the scolytid beetles may be important vectors in the transmission of *Scieroderris lagerbergii* under specific conditions. Wingfield and Marasas (1980) suggested that the fungus-insect relationship of *Ceratocystis ips* and *Orthotomicus erosus* is an important part of a disease complex that results in significant losses in pine plantations. In 1983, the same authors demonstrated the pathogenicity of the root pathogens *Verticicladiella procera* and 2 new species and showed that they were carried by scolytid bark beetles.

Other examples of ambrosia beetles being linked as the vectors of plant pathogens are: the mortality of the Red Beech (*Nothofagus fusca*) which is a direct result of the invasion of the sapwood by a fungal pathogen *Sporothix sp.* introduced by the ambrosia beetle *Platypus sp.* (Faulds ,1977); the sudden death syndrome of cocoa, caused by *Phytophthora palmivora* in Papua New Guinea, which Prior, (1986) linked to the scolytid bark beetles and the ambrosia *Platypus sp.*. In this study Prior found that *F. solani* was also a part of this disease-insect syndrome; and in 1991 Hijii, et. al. suggested that the mass mortality of oak trees in Japan was the result of a range of pathogens introduced by the ambrosia *Platypus quercivorus* and *P. clamus*.

In our current study *F. solani* was consistently isolated from ambrosia beetles sampled. It was also consistently isolated from the dead discoloured bark outside the primary xylem infected with *F. oxysporum*. This bark is characteristically wet, and smells of decay, and is the marker used for locating the *F. oxysporum* infection. This association of ambrosia beetle, *F. solani* and the *F. oxysporum* warrants further investigation.

Of the 132 infected and lightning damaged trees inspected during the last 10 months of the project, 128 (97%) were infested with ambrosia beetle at the time of inspection (**Table 3.1**).

Table 3.1
Classification of the 170 unhealthy trees inspected during the 10 month period November 1993 – August 1994.

		Total unhealthy trees	Trees struck by lightning	Trees struck by lightning	Lightning and ambrosia	Trees and Ambrosia	Ambrosia and infected	Lightning and infected	Lightning and secondary sites	Total unhealthy trees	Total number infected	Secondary sites no lightning	Total number infected	Lightning and infected
Lightning damage	Secondary infection site	103	103	103	100	100	100	103	103	103	103		103	103
Lightning damage	Primary infection site	29	29	29	28	28	28	29		29	29		29	29
Lightning damage	No infection	18	18	18	18	18				18				
No lightning damage	Seconday infection	15				15	15			15	15	15	15	
No lightning damage	No infection	5				5				5				
	Totals	170	150	150	146	166	143	132	103	170	147		147	132
	Percentage		88%		97%		86%		78%		86%	10%		90%

Because of this apparent close relationship between lightning damage, the presence of ambrosia beetles (**Plate 3.10**), and the subsequent infection by *F. oxysporum*, and the demonstrated link of infection by *F. solani* and ambrosia

Plate 3.10
Ambrosia beetles actively invading an Angsana trunk which had recently been struck by lighting.

beetles in the literature, it was important to determine, firstly the identification of the ambrosia beetles and secondly, whether these beetles were capable of carrying *F. oxysporum* as part of their fungal flora.

Collection of ambrosia beetles were carried out between April 1992 and September 1994 at University Road, Holland Road Fringe car park, Pioneer Road and Sembawang Field Experimental Station. The beetles were trapped using Window Flight Traps (Beaver & Loyttyniemi, 1991; Martin, 1977), funnel traps and also collected individually using forcepts.

Whole beetles and severed parts, such as head, thorax and abdomen were analysed for the presence of micro-organisms. The selective media used was a Peptone PCNB agar (PPA) as described by Burgess et. al. (1988). This media was later modified with the addition of 1 g per litre of carbofuran (Furadan® 5G) granules to control the nematodes carried by the beetles, which quickly resulted in bacterial contamination swamping the plates.

3. 2. 1 Identification.

The ambrosia beetle which predominated in all the collections was identified as *Platypus parallelus* (Fabricus) (= *P. linearis Stephens*), Family :Platypodidae. (**Plate 3.11**). This was confirmed by Dr. Mick Cox of the International Institute of Entomology, CAB International, and also by Prof. D. H. Murphy from the National University of Singapore.

The beetle is polyphagous and is found throughout the tropical and subtropical regions of the New World, and in Africa and Malaysia. Dr. Cox (*pers. comm.*) communicated that the beetle could transmit *Fusarium* fungus since the platypodid *Cylindropalpus auricimans* has this ability in West Africa.

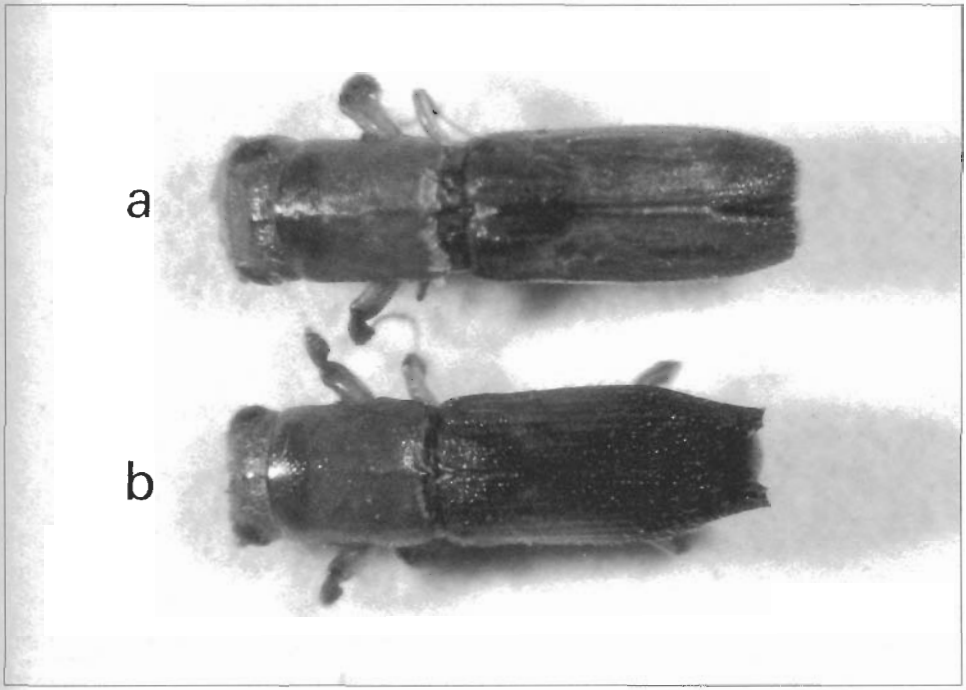
The other species of ambrosia beetle, infrequently caught in the collections, was identified as *Platypus geminatus* by Prof. D. H. Murphy from the National University of Singapore .

3. 2. 2 Isolation of *Fusarium oxysporum* from ambrosia beetle

F. oxysporum, F. solani, Penicillium sp., Gliocladium sp., and *Trichoderma sp.,* were isolated from larvae taken from the galleries. Saprophytic bacteria were also isolated in some cases.

Similarly, the adult insects (male and female) also yielded *F. oxysporum, F. solani, Penicillium sp.,* and *Gliocladium sp.,* and also *Verticillium sp.*

In an effort to localise these micro-organisms, parts of the adult insects were plated and analysed for the presence of fungal growth. *F. solani* was found on the head, thorax and abdomen of the insects, whereas *F. oxysporum* was only detected



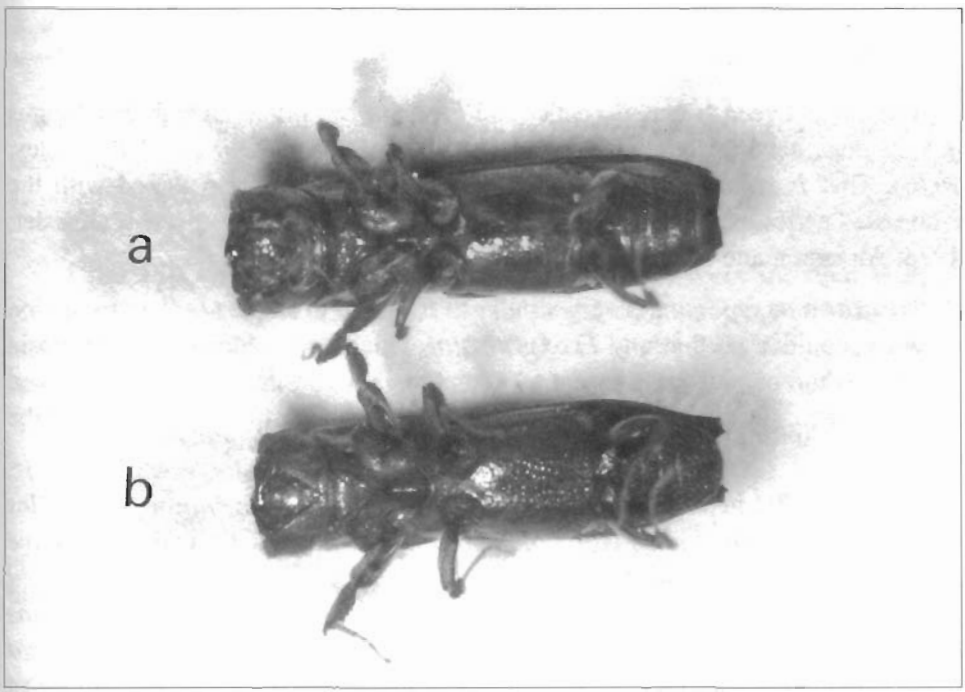


Plate 3.11
The ambrosia beetles (*Platypus parallelus*, family: Platypodidae) trapped from the diseased Angsana. Female (a); Male (b).

on the abdominal parts. *Cladosporium sp.* and *Nigrospora sp.* were also detected in these experiments.

Besides studies on mycangia, other workers were cited by Browne, (1961) who reported that spores could be carried by the beetle on brushes or hairs on the head, under the elytra, and on ventral abdominal hairs. It is also generally agreed that mycelium is utilised as food, but spores usually pass out undigested.

Out of the 200 beetles collected at University Road, only 6% carried *F. oxysporum*, while beetles taken from funnel traps showed 33% had *F. oxysporum* on them. Collections of beetles taken at Holland Road fringe car park, pooled collection of 50 - 100 beetles each, revealed that 30 - 40% of them carried *F. oxysporum*. Of the twenty-six beetles which were either drowned in the resin or captured alive while initiating attack on the bark, 15% were found to carry *F. oxysporum.*

These results demonstrated that where inoculum was high as a result of increased numbers of infected trees, it followed that a larger percentage of the beetles emerging from the area would carry the pathogen. At the university Road there was only one infected tree at the time of trapping, whereas the Holland Road fringe car park area was in the midst of an outbreak. Several trees were diseased or became infected over the trapping period.

3.3 Discussion

The link between ambrosia beetles and *Fusarium solani* as an ambrosia fungus is well documented (Kok, 1972; Zimmermann, 1973; and Hara and Beardsley, 1976). That *F. solani*, an aggressive plant pathogen, was also linked with the ambrosia beetles as a vector of the disease is therefore to be expected. (Kessler, 1974; Anderson and Hoffard, 1978 and Weber, 1979; 1985)

Fusarium oxysporum is very similar to *F. solani* in respect to the production of micro-conidia, so that any *F. oxysporum* which had colonised the ambrosia tunnels, would be picked up by the adult ambrosia beetles leaving the tunnels and transported to, and inoculated into, any new susceptible trees colonised by the beetles.

Murphy (1994 pers. comm.) stated that *"the biology of the ambrosia beetles is centred around the transmission of their fungal symbionts, for which they have specialised anatomical devices such as hair tufts or chitonous pockets designed to carry the spores. If a tree became infected with a fungal pathogen such as has happened in the Angsana wilt disease, it would be almost impossible for ambrosia beetles not to pick it up on emergence, and almost inevitable that they would infect any new tree that they successfully infested themselves."*

Throughout the project it was common when isolating *F. oxysporum*, to find the mycelium to be associated with the ambrosia holes in the wood (**Plate 3.12**),

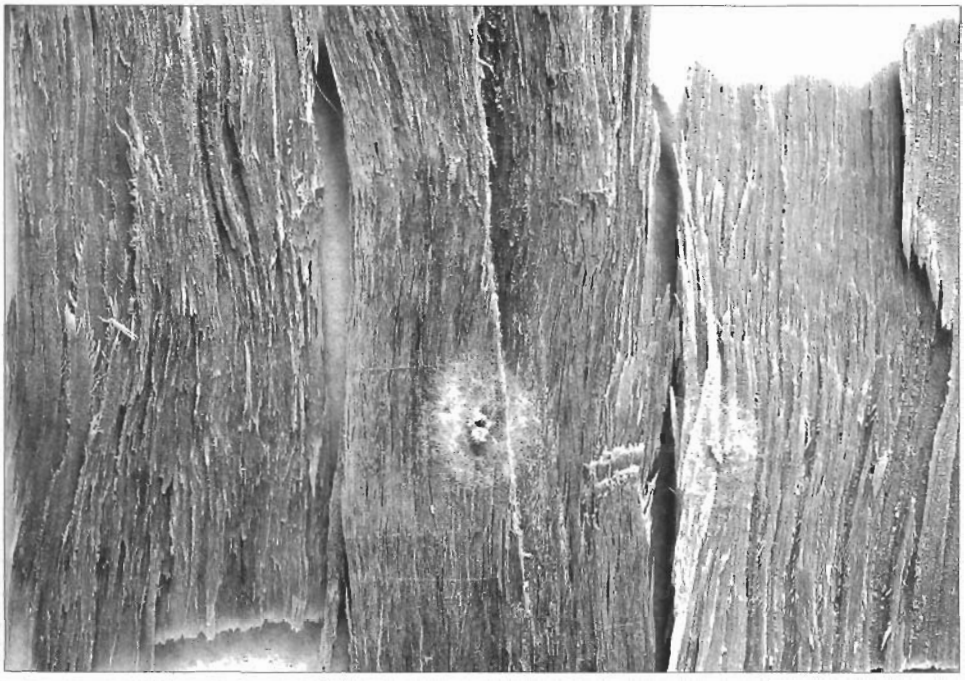


Plate 3.12
White cottony mycelium *F. oxysporum* growing out from Ambrosia beetle holes on surface sterilized diseased wood tissue placed in petri dish. The only mycelial growth to be seen on the wood is associated with the ambrosia tunnel.

however, it was only towards the end of the project that we demonstrated that ambrosia beetles do carry the *F. oxysporum*. In one sample 40% of those beetles sampled were carrying *F. oxysporum*.

Of the 170 unhealthy trees inspected, 147 (86%) were infected with *F. oxysporum*, and of these, 132 (90%) were also struck by lightning and 128 (87%) had both lightning and ambrosia beetles. Only 15 (10%) were due to secondary spread and not related to lightning (**Table 3.1**).

Of the remaining 23 (14%) trees, all were struck by lightning but no *F. oxysporum* was isolated.

These figures demonstrated dramatically the importance of lightning in the epidemiology of the disease, not only in initiating the primary infection sites (97%) but also in the secondary infection sites (86%).

Of the 170 trees examined in the last 10 months of the project, only 4 (3%) of those which had been struck by lightning had no ambrosia beetles associated with the damage at the time of inspection (**Table 3.1**).

The hypothesis presented here for the life cycle of the Angsana wilt disease, is that lightning damage to an Angsana provides the stress which attracts the

ambrosia beetles. If these beetles are contaminated with *F. oxysporum* spores, then infection of the already stressed tree is likely to follow. The secondary spread away from this primary infection site, is by *F. oxysporum* which has entered the soil from the infected tree.

The present epidemic of the Angsana wilt disease is a result of a large build up in the number of infectious ambrosia beetles, and it is this population which should be targeted as the weak link in the disease cycle where, with a combined effort of the various government organisations involved, control of the disease can be achieved.

4. THE CONTROL STRATEGY

Sporadic occurrences of Angsana wilt disease occurred around Singapore between the late 1970s and 1982. This culminated in the visit of Harden (1982) and the following control measures being implemented by Parks and Recreation Department:

- **Removal of diseased trees.**
- **Drenching of the surrounding soil with fungicide.**
- **Horticultural sanitation.**
- **Monitoring.**

With the removal of the infected trees and the intensive soil drenching with fungicides, no further infections were encountered and the spread of the disease appeared to be under control. In October 1983 the Plant Protection Unit of Parks and Recreation Department was disbanded, and no cases of Angsana wilt were recorded between October 1983 and October 1988. In late 1988 two Angsanas along Collyer Quay were found to be diseased. Between 1988 and 1991 over 800 trees died in Singapore because of the disease.

As the implementation of these stringent control measures failed to eradicate the disease, it became one of the primary objectives of the Angsana wilt project to establish possible reasons for the failure, and to formulate a modified disease control strategy, based on the findings of the epidemiological studies.

The control strategy developed in 1982 was based on the assumption that the disease was solely caused by the soil-borne fungus F. oxysporum, and therefore as it was assumed that the disease originated from the soil, there was no urgency to remove infected trees. It was also postulated that drenching the soil with fungicides would eradicate the pathogen from the soil, and also, because the chemicals used were systemic, they would be translocated and therefore have activity against the pathogen within the tree.

The current research, however, has empirically demonstrated that although the disease is of soil-borne origin, the initial spread of the fungus to new sites is by

an insect vector. Controlling the disease once the pathogen has reached the soil in a new site, is therefore to implement control measures at the end of the primary infection cycle. Control, to be successful must be implemented at the beginning of the primary infection cycle.

4. 1 Development Of The Control Strategy

The modified control strategy has two objectives. Firstly to prevent the disease from becoming established in new sites, and secondly to slow down the progression of the disease in those areas where secondary infection is already established.

No control strategy will stop an epidemic instantly. Success depends on slowing down the progress of the epidemic, turning the positive multiplication rate of an active epidemic, into a negative multiplication rate which eventually results in the total control of the disease as the pool of inoculum is gradually reduced to one below epidemic thresholds. Sporadic incidences could then be mopped up.

4. 2 Overview.

There are two main methods of spread of the Angsana wilt disease. Firstly by the *F. oxysporum* infested ambrosia beetles which invade a damaged tree following a lightning strike, and secondly by the traditionally accepted method of soil-borne hyphae and spores, and by root-to-root contact.

The lightning - ambrosia beetle - *F. oxysporum* complex explained 97% of the primary infection sites inspected during the 1993-94 survey. This complex also played a significant role, being associated with 87% of the secondary infection sites (**Table 3.1**).

Most of the pockets of secondary infection sites scattered around Singapore are well established, and the pattern of diseased trees within these pockets suggests that the spread of the disease is predominantly localised. The immediate neighbours of an infected tree are highly at risk with a more than an 80% chance of becoming infected . All Angsanas within 100 m of an infected tree are also at risk, although this distance will vary depending on the terrain between the trees and the assistance this provides for the spread of the fungus.

As a result of the trials on injecting infected trees to stop the development of the disease, it was apparent that once a tree was infected, and subsequently when the symptoms of the disease appeared, it was already too late to control the disease within the tree by chemical treatment. This is understandable as *F. oxysporum* is a vascular pathogen attacking and destroying the vessels which move the fungicides around the tree. Once these vessels are destroyed systemic action of the fungicide stops. Although it was demonstrated that chemical treatment will

delay the onset of the disease symptoms for between 4 - 6 months, it will not prevent the inevitable death of the tree. The fungus will again advance as the fungicide within the healthy vessels becomes diluted or is broken down. Secondly once infection is established, ambrosia beetles rapidly help spread the pathogen within the tree.

The time requirements for the removal of the lightning damaged trees is therefore based on the life cycle of the beetles. This time period, from the first invasion of the tree to the emergence of the second generation of infected beetles can be predicted within narrow limits.

As the ambrosia beetles are the weak link in the disease cycle, and a major contributing factor to the current epidemic, it was pertinent to develop the control strategy around the life cycle of the beetles.

4. 3 The Control Strategy

The control strategy is aimed at breaking the disease cycle by controlling the ambrosia beetles, and thus preventing *F. oxysporum* from infecting the lightning damaged tree and becoming established in the soil. The strategy is based on the rapid removal of these lightning damaged trees, chemical treatments in the form of sprays to prevent insect invasion of the damaged trees and sprays and injections to protect the surrounding trees from invasion and infection.

- **Monitoring.** The success or failure of the control strategy will depend on the efficiency of the monitoring team in detecting the lightning damaged trees early and co-ordinating the treatment programme.

- **Removal of lightning damaged trees.** It is paramount to the success of the control strategy that: 1) insecticide sprays be applied to the damaged tree within days of the **first symptoms of wilting being note**d to prevent the colonizarion by ambrosia beetles, and 2) the tree and as much of the root ball as is possible be removed within three weeks of the **lightning strike**.

- **Treatment of High Risk trees by injection.** All Angsanas within 100 m of an infected tree are considered to be highly at risk and therefore likely to become infected in the future. All high risk trees should be sprayed to run-off as far up the trunk as is practicable, using a conventional horticultural high volume sprayer with a mixture of fungicide and insecticide and injected with a mixture of fungicide and insecticide within three days of the lightning damaged tree being noted. Addition of a sticker to the chemical spray is recommended.

- **Second injection.** Trees should be re-injected 4 months later.

- **Trenching.** Where possible trenching should be carried out between damaged

and undamaged trees to minimize the likelihood of tree to tree spread via root contact.

- **Hygiene.** As with all infectious diseases, hygiene is of paramount importance. All tools which are used on or around infected trees should be treated with 95% alcohol using a hand-held plastic sprayer. Extreme care must be taken to ensure that ambrosia beetles do not get inside vehicles and transported to new areas. Felled trees should be burned to rid them as a source of beetle dispersal.

- **Replanting with Angsanas.** As long as the lighting damaged tree is removed before infection is established within the damaged tree, then there should be no danger in replanting with a second Angsana. However, once infection is established in the damaged tree, then it must be assumed that the fungus has reached the soil and measures taken accordingly.

- **Trees outside the jurisdiction of Government Departments.** Although the success of this control strategy can proceed independently of the removal of lightning damaged trees within private property, owners should be advised that these trees are not only a physical danger to property, but also a health hazard to surrounding Angsanas, and that it is important that these trees be rapidly removed. It would be frustrating to see the control efforts wasted because of one or two small hot-spots of infection remaining within privately controlled land. Hot-spots which would act as the source of infection for subsequent outbreaks of the disease.

4. 4 Discussion

Currently 87% of new infections of Angsana wilt disease are ambrosia beetle related and it is this population of infectious beetles which is the initial target of the control strategy. To control this population is to control the epidemic. This can only be done by the rapid removal of all lightning damaged trees, the potential sites of infection. Initially, this will therefore mean the removal of 10% of trees which would not have become infected, however, this is a small price to pay for the ultimate success of the control strategy. As the control strategy takes effect and the population of infectious ambrosia beetles has reduced then the number of trees likely to remain healthy will rise to an ultimate aim of 100%. As the population of infectious ambrosia beetles drops, decisions will have to be made as each new lighting damaged tree is detected, as to whether or not the tree should be removed on the basis of the likelihood of it becoming infected.

Following the implementation of the control strategy, there will be a period of several months before there is a reduction in the number of trees being removed due to Angsana wilt each month. This lag period will correspond to the time that it takes for all those trees which are already infected at the time of treatment, to develop symptoms and be removed. Once this reservoir is removed and the

population of infectious ambrosia beetles is reduced, rapid progress should be made in controlling this insidious disease.

5 SCREENING OF ANGSANA (*Pterocarpus indicus*) FOR RESISTANCE TO *Fusarium oxysporum*

Although the short term aim of the project was to develop a control strategy based on chemical control, the long term aim was to identify lines of *P. indicus* resistant to *F. oxysporum*.

5.1 Collection of planting material

Planting material was collected from four localities in West Malaysia, ten localities in the Philippines, three in Myanmar, five in the Solomon Islands, three in Papua New Guinea, three in Java, eight in the Sulawesi Islands and six localities in the Maluku Islands. In total, 610 trees were sampled for hardwood cuttings and 188 samples of seeds were collected (**Fig. 1**).

Pterocarpus indicus is a natural component of the coastal low-land flora of South East Asia, however, because it is prized as a timber for both building and for furniture, it is very rare to see mature trees in the jungle accessible to the local population. Conversely, because it is so easily propagated and provides excellent shade, Angsanas were commonly found growing in villages as either single trees or as avenues for shade trees, and as rows of trees for fencing. It was also found as isolated shade trees in cleared jungle being used for agriculture.

Planting material was also collected from the Angsanas growing around Singapore on a weekly basis between February and November 1993. During which time 270 samples of cuttings and 58 collections of seed were made.

Where possible 100 seeds per tree were collected (**Plate 5.1**) as, not only were they physically easier to collect and gave a greater establishment rate, but it also meant that we were selecting within a segregating population where each seedling was genetically unique.

Where seeds were not available, 3 or 4 hardwood cuttings, 20 - 25 cm long and between 2 - 3 cm in diameter were cut. Hardwood cuttings were collected by removing a 2 - 3 cm diameter branch, using either a pruning saw or a tree pruner which was extendible to 5 m. The branch was then cut into 3 - 4, 20 cm lengths. These were placed into a zip-lock® plastic bag for transport. Data were recorded on tree locality and physical characteristics, such as tree form, leaf shape and size etc. Cuttings were soaked in water overnight, then soaked for 30 min in a 1:10 sodium hypochlorite solution (Chlorox®), wrapped in paper towels treated with Benomyl (Benlate® 50WP), and returned to the zip-lock® plastic bags for transport. Every effort was made to ensure that all hardwood cuttings were planted into soil within 14 days from collection.

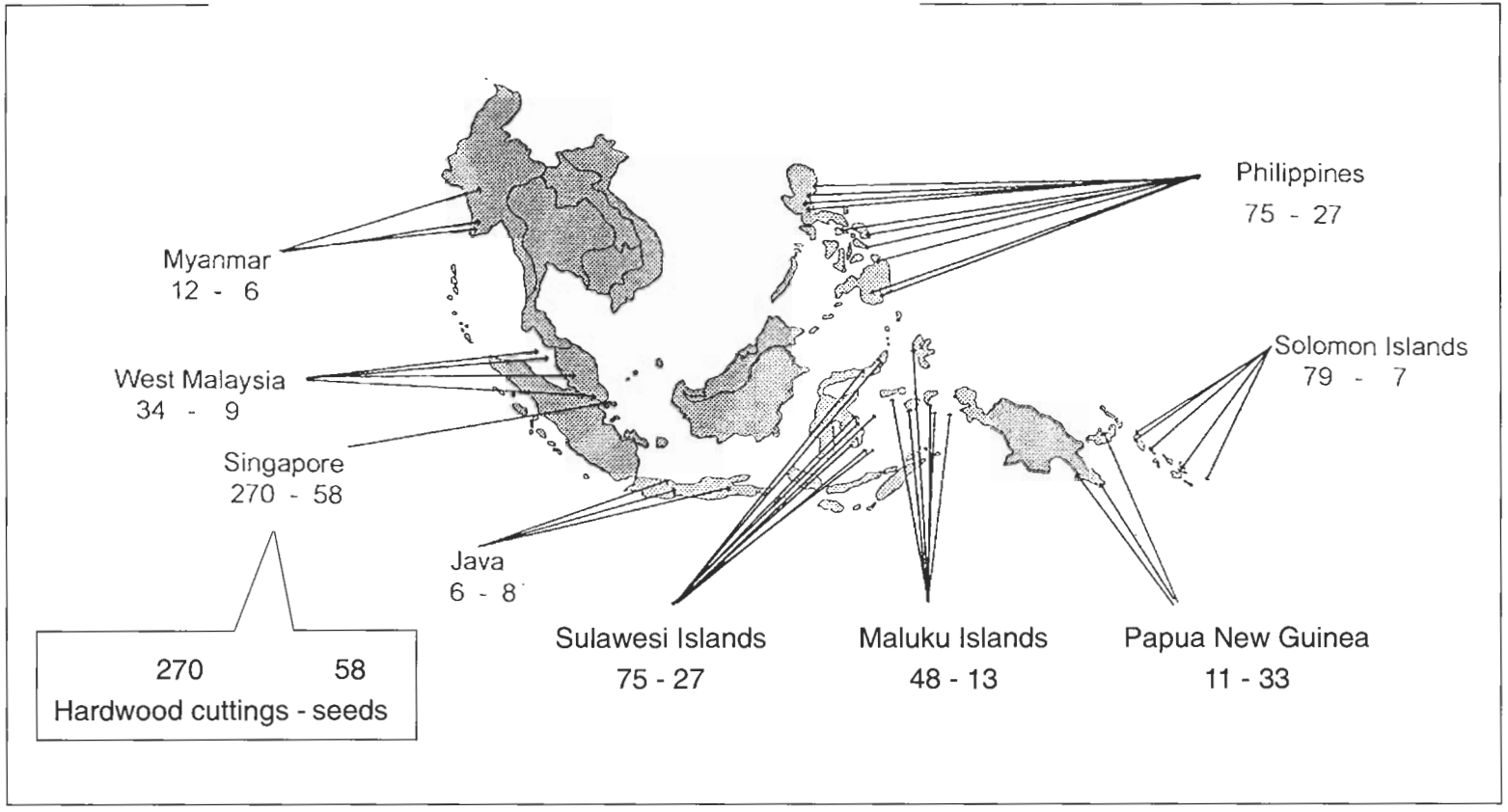


Fig. 1 Collection of hardwork cuttings and seeds

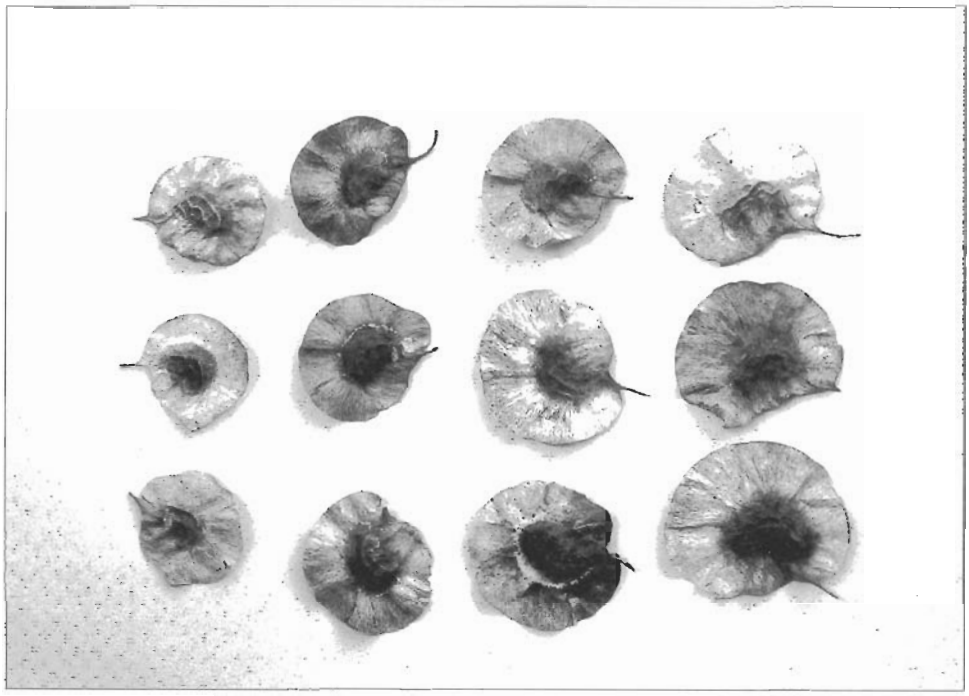


Plate 5.1
Angsana seeds collected from both Singapore and overseas exhibit a large variation in size across the wing, from 3.5 cm to 7 cm.

In instances where neither seeds nor hardwood cuttings were available, then softwood cuttings were taken.

The wings of the seeds of *Pterocarpus indicus* were cut to expose the seeds inside the pods. Seed pods were soaked in water overnight before being sown in washed coarse sand in 450 × 345 × 70 mm plastic trays. Fresh seeds usually germinated within three days. However, this period is extremely variable with some seeds germinated overnight while a few exceptions took over a year to germinate. Vigorous seedlings were pricked out into 75 mm plastic pots containing a general potting mix. Seedlings were allowed to grow until they were between 10 - 15 cm tall before being screened for resistance.

Hardwood cuttings when they arrived from overseas, were thoroughly cleaned and soaked in a dilute fungicide solution for 30 min. before being potted in 75 or 120 mm plastic pots in either sand, soil or a general potting mix . The cuttings were allowed to grow until they were well rooted and growing vigorously before being put through the screening process. The establishment of hardwood cuttings depended on the time from cutting to planting. The establishment rate for the Singapore cuttings was 62% while the establishment of those collected overseas ranged from 13% to 19%.

5. 2 Screening for Resistance

A weekly cycle was developed to enable an efficient screening of the large number of established hardwood cuttings and seedlings established in the nursery.

Five different isolates of *F. oxysporum* were grown on PDA (Potato Dextrose Agar) at 28°C for 5 days. Five plugs were removed using a sterilised 5 mm cork borer and ground in a 10 ml glass macerator to form a thick suspension. This suspension was added to 500 ml of PDA at 45 - 50°C just prior to pouring. The petri-dishes were left on a bench in natural light to stimulate the production of micro-conidia. After seven days the fungal lawns were blended together in sterile water. The final concentration used was three plates to 500 ml of sterile water.

Cuttings and seedlings were removed from their pots, labelled, and their roots washed clean and trimmed to about 1.0 - 1.5 cm in length. They were then placed into plastic jars containing the inoculum. They were left in the inoculum for four hours under full sunlight, before being re-potted using the original potting mix. The pots were then watered with the inoculum from the jars. The inoculated cuttings or seedlings were only watered when the soil became dry in order to maintain them under mild stress. Inoculated plants were monitored closely for five weeks, and plant deaths were recorded accordingly.

5. 3 Results

Inoculated plants usually started to show signs of wilting (yellowing and dropping of leaves) towards the end of the third week. Scraping the stem to reveal some discoloration and vascular staining would confirm that the material was infected. Some plants died during the third and fourth weeks, however, the majority of plants died during the fifth week, with a few taking more than five weeks to wilt and die.

At the end of five weeks, those plants that had survived were checked, and healthy plants were re-labelled and returned to the nursery area to await subsequent screening.

From November 1992 to Nov. 1995, 35 batches of *P. indicus*, consisting of over 2,600 seedlings and 650 cuttings were subjected to one screening, and from those, 1,214 seedlings and 69 cuttings have since been inoculated the second time, and 652 seedlings and 47 cuttings were inoculated for the third time.

All plants will now be planted into natural soil known to contain *F. oxysporum*, for a final confirmation of resistance.

5. 4 Discussion

During the course of the project over 650 hardwood cuttings and 2,600

seedlings which had been collected from 7 countries were tested. Because we are looking for a single gene resistance, which is expressed as an all-or-nothing reaction, resistant plants survive and susceptible plants die.

All the material had been subjected to three screenings. All surviving plants will now be planted into natural soil which is known to contain *F. oxysporum* before we can confidently describe this material as resistant to *F. oxysporum*.

From **Table 5.1** it can be seen that there is a wide range in the percentage survival of the hardwood cuttings. This is understandable as the resistance/susceptibility of the hardwood cuttings is that of the parent tree. The low incidence of survival of hardwood cuttings from Singapore (25%) would suggest a low incidence of resistance within the Singapore Angsana population. This could go some way to explain the development of the disease here rather than in other regions.

Table 5.1 Screening of Angsana seedlings and hardwood cuttings for resistance to *Fusarium oxysporum*.

	SEEDLINGS								
	First inoculation			Second inoculation			Third inoculation		
ORIGIN	Number inoculated	Number survived	Percentage survived	Number inoculated	Number survived	Percentage survived	Number inoculated	Number survived	Percentage survived
Indonesia	105	38	36	27	20	74	11	11	100
Malaysia	232	72	31	68	50	74	19	16	84
Myanmar	166	81	49	81	56	69	19	18	95
Papua New Guinea	350	180	51	166	119	72	35	27	77
Philippines	1,256	824	66	684	591	86	484	461	95
Singapore	528	240	45	169	146	86	79	79	100
Solomon Islands	57	20	35	19	17	89	5	5	100
TOTALS	2,694	1,455	54	1,214	999	82	652	617	95

	HARDWOOD CUTTINGS								
	First inoculation			Second inoculation			Third inoculation		
ORIGIN	Number inoculated	Number survived	Percentage survived	Number inoculated	Number survived	Percentage survived	Number inoculated	Number survived	Percentage survived
Indonesia	51	28	55	12	11	90	4	2	50
Malaysia	17	10	59	2	2	100	1	0	0
Myanmar	9	3	33	2	2	100	—	—	—
Papua New Guinea	14	3	21	—	—	—	—	—	—
Philippines	21	11	52	2	2	100	2	0	0
Singapore	483	121	25	47	32	68	38	25	66
Solomon Islands	71	19	27	4	3	75	2	2	100
TOTALS	666	195	29	69	52	75	47	29	62

The ratio of resistance to susceptible plants in the seedling population must, however, reflect the genetic composition of the population. That the overall percentage of survival was close to 50% (535) is encouraging, and further genetical studies will be carried out.

That the survival of the seedlings increased from 54% in the first inoculation to 82% in the second and to 95% in the third inoculation is an encouraging indication that resistance has been identified. On the other hand, at no time did the results obtained with the hardwood cuttings parallel the success obtained with seedlings. Not only was the initial establishment of the hardwood cuttings very low (less than 20% for overseas cuttings) but the attrition rate while growing the plants in small plastic bags under the direct tropical sun was very high.

This work will continue and it will take an estimated further two years to have resistant planting material available for multiplication.

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