STUDIES ON THE BLAST DISEASE OF SEEDLINGS OF THE OIL PALM (ELAEIS GUINEENSIS JACQ.)

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by

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SECTION I

ABSTRACT

The series of investigations carried out into the blast disease of oil palm seedlings, a disease of high economic importance, showed that the disease was widespread in nurseries throughout south-western Nigeria. The disease was prevalent between October of the year of planting and March of the following year and appeared to be severest in November and December. The incidence of the disease was lower among seedlings planted into the nursery at the beginning of the rainy season in April than among seedlings planted in June, July or August. Coconut (Cocos nucifera Linn.) and other palms of economic and ornamental importance, for example, Areca catechu Linn., Ptychosperma elegans Blume, and Roystonea regia (H. B. K.) Cook also showed heavy blast infection.

Pythium splendens Braun (Accession No. IMI 149554) and Rhizoctonia lamellifera Small (Accession No. IMI 149556) were consistently isolated from infected roots. Pathogenicity tests with these fungi showed that the blast disease developed as a result of the co-infection of oil palm seedlings by both fungi. P. splendens was found to be more pathogenic than R. lamellifera under experimental conditions.

The infective propagules of P. splendens were found to be more abundant at the ploughline depth of 9 inches than at depths of 1, 3, 6, 12, 15, 18, 24 and 36 inches. The recovery of the fungus from nursery soil by the root baiting technique was high in July-October and low in November-January. These variations in the recovery of the fungus from soil were found to correspond with variations in soil moisture but not with soil temperature. The cultural practice of incorporating organic manure with soil increased while air-drying of soil decreased the recovery value of the fungus from soil.

In vitro studies on *P. splendens* and *R. lamellifera* showed that the linear growth, sporangial and sclerotial production and germination were better in agar media rich in simple sugars than those deficient in them. The best temperature range for the linear growth, sporangial and sclerotial production and germination was found to be 25-30°C. Longevity of sclerotia of *R. lamellifera* was highest at a relative humidity value of 95% and at a low temperature of 20°C.

Soil temperature in polythene bag planting was found to be positively correlated ($r = 0.7758$) with the blast disease at the 1% level of significance. There was no statistically significant correlation between the disease and soil temperature or soil moisture in ground bed planting. The incidence of the blast disease was found to be higher in the subsoil and ground bed planting than in the topsoil and polythene bag planting respectively.

Shading of nursery seedlings in the dry season particularly from October to December was found to be more effective than chemical soil treatment with Fernasan or Benlate in reducing the incidence of the blast disease. Soil treatment with pentachloro-nitrobenzene appeared to aggravate the disease. Extension work seedlings produced at NIFOR were found to be more resistant to the blast disease than grove palm seedlings.

SECTION II

INTRODUCTION

The oil palm (Elaeis guineensis Jacq.) is one of the most important economic plantation crops in the Rain Forest Zone of Southern Nigeria. It is also cultivated in many parts of the humid tropics, notably Malaysia, Indonesia, Cameroun and the Ivory Coast, throughout the world (Anon., 1970b). About 162,802 acres of oil palm are cultivated in Nigeria and wild oil palm covers another 5,950,000 acres (Ndaeyo et al., 1971). Palm oil, palm kernel and palm kernel oil produced from these palms accounted for about £14.4 million in foreign exchange for Nigeria in 1970 (Ndaeyo et al., 1971).

It is believed that the original home of the oil palm is West Africa (Hartley, 1967). Throughout West Africa, the oil palm grows wild in natural groves found mainly in a belt of 200 - 300 miles wide along the coast from Gambia to Angola (Moe and Mohtadi, 1971). In recent years, attempts were made in Nigeria, Zaire (formerly Congo-Kinshasa), Cameroun and the Ivory Coast to establish commercial plantations of improved, high-yielding varieties of the oil palm to replace the low-yielding wild palms. The oil palm grove rehabilitation scheme was introduced during the 1962-1968 Development Plan period by the former Eastern Region of Nigeria. Unfortunately, a significant proportion of the rehabilitated palms was destroyed or set back during the 1967-1969 Nigerian civil war (Anon., 1971b). The result is that the bulk of Nigeria's

palm produce (palm oil and palm kernel) is still derived from wild or semi-wild palms (Anon., 1970b). In 1963, for example, the proportion of palm oil contributed by commercial plantations was only 6% of the total palm oil exported (Forde, 1969).

Prior to 1966, Nigeria was the world's premier producer of palm oil and palm kernel (Anon., 1970b). During the 1959-1965 period, her average annual export of 163,000 tons constituted about 30% of world trade in palm oil (Forde, 1969). Nigeria's average annual export of palm kernel during the same period was 414,000 tons which was about 50% of world trade in that commodity (Forde, 1969).

In 1966, however, Nigeria's commercial production of palm oil (130,000 tons) was surpassed by that of West Malaysia (183,000 tons) which has now become the world's leading producer of palm oil (Anon., 1970b).

The civil war in Nigeria resulted in a significant decrease in the commercial production of palm oil. Palm kernel supplies from Nigeria were, however, not as severely affected by the conflict as those of palm oil. Despite a fall in the production of palm kernel during the civil war, Nigeria continued to be the world's major producer of palm kernel (Anon., 1970b). This was because a large proportion of the exported palm kernel was produced in the Western State of Nigeria which was not seriously affected by the civil war.

As a foreign exchange earner, palm produce alone, in 1900, accounted for over £1.5 million or 81.6% out of a total contribution of 95.6% made by agricultural commodities to the Nigerian economy (Forde, 1969). This trend in the relative proportion of oil palm contributions to earnings from agricultural commodities was maintained for nearly twenty-five years during which period the contributions of cocoa and groundnuts were becoming increasingly important. Around 1925, palm produce accounted for 53.7% of total export revenue (Forde, 1969). Between 1925 and 1955, the relative importance of palm produce dropped to about 30% and during the period 1960-1965, it varied between 15% and 24% of total earning from agricultural export (Oyenuga, 1967).

Palm oil and palm kernel oil are used industrially in the manufacture of margarine, compound cooking fats, soap, candles, cosmetics, confectioneries and as a lubricant in tin-plating (Forde, 1969). Palm kernel cake, a by-product from the extraction of palm kernel oil, contains about 20% protein and is widely used as livestock feed (Oyenuga, 1959).

Throughout West Africa, palm oil is used in the preparation of various dishes such as stew and vegetable soup. The consumption of palm oil in Nigeria has been estimated at 150,000-200,000 tons a year by Hartley (1967). More recently, domestic consumption has been estimated at 200,000-300,000 tons a year (Anon., 1970b). Palm oil is rich in carotene from which vitamin A is derived and it is felt that its consumption helps to reduce the incidence

of vitamin A deficiencies (Forde, 1969).

The oil palm also provides palm wine, a popular Nigerian drink which can be converted to vinegar or acetic acid. The latter product is an important coagulant in the rubber industry.

Other local uses of the oil palm include the use of the leaves as thatch for houses, the rachis for fencing and reinforcing buildings and the mid-ribs of the leaflets for making brooms. Oil palm bunch refuse is still used in the southern States of Nigeria for making soap. The fibres and shell which are by-products of palm oil and palm kernel extraction are useful as fuel and aggregate materials for flooring.

It is estimated that by 1980, the annual consumption of palm oil in Nigeria would have risen to 831,000 tons (Ogor, 1969). Export earnings from palm produce in the same year are expected to reach a figure of £300 million (Anon., 1971b). In order to meet both domestic and export demands by 1980, it is projected that a total land area of 5,202,200 acres (made up of 1,207,200 acres of new plantations and 3,995,000 acres of palm groves) have to be under palms (Anon., 1971b). Only 162,802 acres of new plantations have, up to 1971, been established (Anon., 1971b). It is envisaged that the remaining 1,044,398 acres would be planted within the next 9 years and this signifies the estimated extent of planting activity during the next decade. Nearly 63 million seedlings will be required for such a field planting programme

and such seedlings have to be raised first in nurseries.

The most serious threat to the successful establishment of oil palm nurseries is a root disease commonly called "blast". It is essentially a nursery disease and causes the death of seedlings. Seedling losses due to the blast disease in Nigeria are about 8-10% annually and in some years, losses may be as high as 20% (Robertson, 1959a). Heavy losses of valuable nursery seedlings result in considerable financial losses and inevitable delay in field planting programmes.

At a research station, such as the Nigerian Institute for Oil Palm Research (NIFOR formerly WAIFOR - West African Institute for Oil Palm Research), near Benin City, a high seedling mortality due to the blast disease could jeopardise the breeding programme. It could also delay the production of improved, high-yielding hybrid seeds (extension work seeds). NIFOR sells these seeds, in the first instance, to the States' Ministries of Agriculture which, in turn, sell the seeds at low, government-subsidised prices to farmers. Seeds which are specially selected for resistance to the blast disease are unfortunately not yet available. The work on resistant varieties to 'blast' is slow because of the slow growth of the oil palm.

In the NIFOR programme of research and production of hybrid seeds and seedlings for the States' Ministries of Agriculture and the farmers, a minimal loss of seedlings due to the blast disease is imperative.

The aim of this investigation is to study, in detail, the distribution, biology and ecology of the disease organisms, and in particular the factors that influence the development and severity of the disease, and to utilise this knowledge in devising effective and practical methods of control of the disease.

SECTION III

REVIEW OF LITERATURE

1·0 The oil palm blast disease - historical background.

The term "blast" was first used by Trueblood in 1944 to describe a disease of the oil palm because seedlings affected by the disease appeared to have been burnt by fire (Anon., 1953). Other workers have subsequently given other names to the disease. West and Ferguson, cited by Bull (1954), called it "Seedling Wilt Disease" while Waterston, also cited by Bull (1954), referred to the disease as "Cortical Root Rot". Out of the many names given to the disease, "blast" which is the one most commonly used in the literature has been adopted in this study.

The blast disease of the oil palm was first observed and described in Nigeria by Trueblood in 1944 (cited by Robertson and Bull, 1956; Robertson, 1959a). It is essentially a root disease of the oil palm seedlings in planted nurseries. Occasionally, however, the disease was observed in backward palms in planted fields and natural groves (Robertson, 1959a; Robertson et al., 1968). About 95% of the oil palm nursery seedlings attacked by blast succumbed to the disease (Robertson and Bull, 1956; Robertson, 1959a; Robertson et al., 1968). These authors also reported that the surviving 5% were usually malformed or too retarded in size and vigour to be used for field planting.

In Nigeria, the incidence of the blast disease varies with seasons and localities. In some seasons, Robertson (1959a) recorded losses as high as 20% in nurseries at the Nigerian Institute for Oil Palm Research (NIFOR) Main Station, near Benin City in the Midwestern State of Nigeria. Between 1949 and 1959, the annual loss of seedlings due to the blast disease in oil palm nurseries throughout Nigeria was about 8-10% (Robertson, 1959a). The blast disease has also been observed in oil palm nurseries at Ogba Farm in the Midwestern State, Abak in the South-Eastern State and other oil palm nurseries throughout the country (Robertson, 1959a).

Outside Nigeria, the blast disease has been reported from the Cameroun, Ghana (formerly Gold Coast), Sierra Leone, the Ivory Coast, Dahomey and Zaire (Anon., 1953 and Bachy, 1958). In these countries, oil palm seedling losses due to the disease were often as high or higher than 50% (Robertson and Bull, 1956; Bachy, 1958; Robertson, 1959a) In the Ivory Coast, for example, Bachy (1958) reported that more than 80% of the seedlings in a nursery may succumb to the blast disease. In these countries, therefore, the disease constitutes a serious threat to the successful establishment of oil palm nurseries.

It appeared that the earlier records of the blast disease were from oil palm-growing areas of West Africa. In 1965, however, the disease was reported, for the first time, in Malaysia (Turner, 1965; Turner, 1966;

Turner and Bull, 1967). In a severe outbreak of the disease in an estate nursery in Malacca State, 20-25% out of a total number of 6,000 seedlings planted were affected (Turner, 1965). A seedling root rot (presumably synonymous with the blast disease) of the oil palm has also been reported from Sabah by Williams, cited by Turner and Bull (1967).

Although associated mainly with the oil palm, the blast disease has also caused seedling losses in other palms such as Corozo oleifera (H. B.K.) Bailey, Areca catechu Linn., Euterpe Cocos nucifera Linn. and Roystonea oleracea (Mart.) Cook at the NIFOR Main Station (Robertson et al., 1968).

2.0 Symptoms of the oil palm blast disease.

The symptoms of the blast disease have been described by Bull (1954), Robertson and Bull (1956), Robertson (1959a and 1959b) and Robertson et al. (1968). These authors reported that the first visible symptom of the blast disease is the disappearance of the gloss which is characteristic of healthy leaves. The leaves of infected seedlings become dull and flaccid; their colour changes to olive-green, then to greenish yellow. The leaf tips turn purple in colour and chlorosis, followed by necrosis, gradually affect the whole leaf surface which becomes dark-brown and brittle (Plate 1). Leaf tissues surrounding discrete Cercospora leaf spots may remain green for some time so that in oil palm seedlings affected by the blast disease and Cercospora leaf spot, the lesions of the latter disease appear as groups of dark-brown

Plate 1. Two oil palm seedlings (6 months old) in the nursery at the NIFOR Main Station. Healthy seedling (A). Seedling affected by the blast disease (B).

spots situated on green "islands" on a general back-ground of purplish brown leaf.

Most of the seedlings affected by blast also show a necrosis of the central spear leaf. This necrosis affects either the tip of the unexpanded spear leaf or only the basal tissues. The growing points of affected seedlings are not usually destroyed.

The authors further reported that the fact that blast is caused primarily by a root infection becomes obvious when seedlings showing only the initial leaf symptoms of the disease are pulled up to reveal a root system in an advanced state of decay. Root infection is found to occur at the tips of the fleshy roots where the parenchymatous tissues within the hypodermis are rapidly destroyed leaving only the stele within a hollow cylinder of hypodermis. A diseased oil palm seedling if pulled up shows only the naked vascular strands (Plate 2). At the initial stage of infection when a soft, wet rot of the cortex is to be found advancing along the roots, the transition zone between healthy and infected tissues is not clearly defined but the zone becomes distinct as infection progresses into older root tissues. In a longitudinal section, the transition zone appears between brown, water-soaked, necrotic tissues and healthy cortical tissues. In some infected roots, the rotted tissue dries to a greyish-blue colour while occasionally the transition zone is marked by a broad, yellow band delimited proximally by a brown line.

Plate 2. Root system of 6 month-old oil palm seedlings in the nursery. Healthy seedling (A). Seedling affected by the blast disease (B). Note the naked vascular strands

Externally, the transition zone is not discernible but where infection has reached an advanced stage, such a zone is visible as a dark-brown or black discoloured zone. The rot progresses along the roots until it reaches the stem tissues or bulb where it stops. In a longitudinal section, the tissues between the rotten roots and spear leaf of a diseased seedling are usually healthy, although it is not clear why the bulb is not affected. There is no evidence of a physical barrier to organisms and it is, therefore, probable that resistance of the bulb to invasion is physiological.

The vascular strands which remain after the cortical tissues have been destroyed as well as the inner surfaces of the hypodermis are often covered with numerous, minute, brownish-black sclerotia sometimes visible to the eye.

3.0 Aetiology of the oil palm blast disease.

Although the blast disease of oil palm was first reported and described in 1944, very little was known about its causal organisms. Prior to 1954, investigators working on the blast disease in Nigeria and Cameroun always associated some nematodes with the disease (Robertson, 1959a). The nematodes which they observed most commonly were Aphelenchus avenae Bastian and Cephalobus sp.. On the other hand, Zeldia sp., Ditylenchus sp. and Acrobeloides sp. were less frequently isolated (Robertson and Bull, 1956; Robertson, 1959a). These were all saprobic nematodes and the workers

were unable to prove, in pathogenicity experiments, that the organisms were the causal agents of the disease.

Goodey in 1957, cited by Robertson (1959a), isolated a parasitic form of nematode, Hoplolaimus proporicus Goodey, from root samples of diseased oil palm seedlings sent to the Rothamsted Experimental Station by the Commonwealth Development Corporation. He, however, did not consider that the nematode was present in sufficient numbers to account for the extensive root damage observed (Robertson and Bull, 1956; Robertson, 1959a).

Fungi were also observed to be associated with diseased oil palm seedlings. Robertson and Bull (1956) and Robertson (1959a) reported the association of the following fungi with the diseased oil palm seedlings:

Fusarium solani sensu Snyder and Hansen

Fusarium oxysporum Schl.

Other Fusarium spp.

Penicillium wortmanni Klocker

Helminthosporium sp.

Curvularia lunata (Wakker) Boedijn.

Glomerella cingulata (Stonem) Spauld and von Schrenk.

Nigrospora sp.

Cladosporium sp.

Absidia crista Dade.

Cephalosporium sp.

Aspergillus violacea-fuscus Gasp.

Rhizoctonia lamellifera Small

Inoculation experiments with most of these fungi to determine if any of them was the causal organism of the blast disease of the oil palm gave very inconsistent and inconclusive results.

Because Pythium sp. and Rhizoctonia lamellifera were more frequently and consistently isolated from infected roots, a greater number of inoculation experiments with these fungi was carried out. From these experiments, Robertson (1959b) concluded that the blast disease developed as a result of the co-infection of oil palm seedlings by the two fungi. He stated further that Pythium sp. was the primary pathogen which penetrated healthy undamaged roots at the growing points and that R. lamellifera, as a secondary invader, played an important role in the destruction of cortical tissues after first establishing itself by parasitizing Pythium sp. He reported that the characteristic leaf symptoms of the blast disease were due partly to drought, resulting from the disruption of the seedling-moisture relationship due to root destruction, and partly to the effect of fungal toxins produced in the roots by Pythium sp. and translocated to the leaves (Robertson and Bull, 1956; Robertson, 1959a).

Most of the studies on the aetiology of the blast disease have been carried out in Nigeria. In Malaysia, also, a species of Pythium was reported to be associated with the blast disease and R. lamellifera has been isolated from infected roots (Turner and Bull, 1967). The report of these two workers gave no information on the role of these two fungi in the development of the blast disease.

4.0 The biology of Pythium sp. (probably P. splendens Braun)

The genus Pythium is cosmopolitan in occurrence both as a soil inhabitant (Garrett, 1960 and 1970) and as an incitant of root diseases of a wide variety of plants (Waterhouse and Waterston, 1966). Wheeler (1969) considered P. splendens to be one of the most important ten species of the genus. P. splendens occurs in the warm temperate and tropical areas of Africa, Asia, Australasia and Oceania, Europe, North and Central America (Waterhouse and Waterston, 1966). In Africa, the fungus has been reported from the Ivory Coast, Madagascar, Nigeria, South Africa and Tanzania (Waterhouse and Waterston, 1966; Robertson, 1959a).

In addition to the blast disease of the oil palm, P. splendens is also known to cause the root rot of maize, pawpaw, slash pine, aroids, aloe, Easter lily, wilt of betel pepper, crown rot of rhubarb, black stem rot of pelargonium and mottle necrosis of sweet potato (Waterhouse and Waterston, 1966). In Hawaii, the fungus is associated with the wilt disease of pineapples

(Sideris and Paxton, 1931; Sideris, 1932). The fungus also causes the root rot of safflower (Zimmer and Thomas, 1969; Klisiewicz, 1968) and has been shown to be pathogenic to barley and oats (Kilpatrick, 1968).

The hyphae of the fungus penetrated directly through the cell walls into the tissues of the oil palm root, grew intra-cellularly within the root and caused the collapse and disintegration of cell protoplasm (Robertson, 1959a).

It is not easy to isolate Pythium spp. from soil by the usual soil-dilution and soil-plate techniques. The most frequently used technique for isolating Pythium species from soil and infected roots is by means of selective media. Meredith (1940), McLaughlin (1947) and Warcup (1957), however, successfully isolated the fungus from soil without the aid of antibiotics. Thus agar media amended with aureomycin (Harrison, 1955), agri-mycin (Singh and Mitchell, 1961), endomycin (Schmitthenner, 1962), Mycostatin (Vaartaja and Bumbieris, 1964; Kerr, 1963; Hendrix et al., 1966; Vaartaja, 1967), pimaricin (Eckert and Tsao, 1960; Klemmer and Nakano, 1962; Vaartaja, 1968) have been used for qualitative and quantitative studies on Pythium species from the soil.

For qualitative studies alone, different plant and animal parts have been used as baits for isolating pythiaceous fungi from soil. Hemp seeds, flies, worms, ants, cockroach wings, potato slabs (Butler in 1907, cited by Rajagopalan and Ramakrishnan, 1963), pineapple leaf bases and roots (Klemmer

and Nakano, 1962) lemon fruits (Klotz and DeWolfe, 1958), cucumber fruits (Banihashemi, 1970), have also been successfully used. Hine and Luna (1963) using freshly diced potato cubes soaked in 100 p.p.m. pimaricin combined the baiting technique with antibiotics for the selective isolation of Pythium aphanidermatum (Edson) Fitzpatrick from soil.

The literature on the isolation of P. splendens from soil or infected roots is meagre. Although most of the general techniques might suffice, the "plate drop" method used by Stanghellini and Hancock (1970) appears to be the most recent and is reported to be efficient and to give reproducible results. The technique was specifically developed for isolating Pythium ultimum Trow. from soil, but it could probably be adapted for studying P. splendens.

In his studies on the blast disease, Robertson (1959a and 1959b) isolated Pythium sp. (probably P. splendens) directly from only primary infections of the root tips. He was also able to demonstrate the presence of the fungus in infected roots in an advanced state of decay by means of the Hopkin's washing technique (cited by Robertson, 1959a and 1959b).

In physiological studies, Robertson (1959a) found that the fungus grew best at a temperature of 29°C and a soil moisture content of 10%. At higher soil moisture, growth was reduced. Waterhouse and Waterston (1966) reported that P. splendens grew at a moderate rate of 20 mm in 24 hours at 27-32°C in an unspecified agar medium and found that the minimum, optimum and

maximum temperatures for mycelial growth were 4°C, 30°C and 37°C respectively.

Parasitism of P. splendens by another fungus was reported by Butler (1957) who found that Rhizoctonia solani Kühn actively parasitized P. splendens when the two fungi were grown in a mixed culture. Robertson (1959b), in a similar study, observed that the hyphae of R. lamellifera penetrated those of Pythium sp. (isolated from blasted oil palm seedlings) after approximately 72 hours of coming into contact and that the former fungus caused an immediate lysis of the cell contents of the latter fungus. Penetration of sporangia was either direct or multiple where hyphae of R. lamellifera became grouped to form a cluster of appressoria around a sporangium prior to infection. The cytoplasm within invaded sporangia disintegrated and disappeared resulting in the ultimate collapse of the sporangia.

4.1 Rhizoctonia lamellifera Small

R. lamellifera Small occurs as a soil inhabitant in sub-tropical and tropical areas of the world, notably Palestine, Egypt, West Africa, East Africa, Ceylon, Formosa, the Philippines and the western States of the United States of America (Robertson and Bull, 1956).

Robertson (1959a) has reviewed the controversy in the literature as to whether R. lamellifera was an active parasite of plants or not. There were three schools of thought. In the first group were workers including Small in

1927-1928 (cited by Robertson, 1959a) who considered R. lamellifera to be an active parasite capable of penetrating healthy tissues and causing a rapid degeneration of both cortical and vascular tissues. Investigators in the second group including Briton-Jones in 1928, also cited by Robertson (1959a), asserted that the fungus was a facultative root parasite and that penetration into the host was dependent on certain conditions predisposing the host to infection. The third group of workers considered the fungus to be a secondary parasite or a saprophyte.

R. lamellifera was first described from its pycnidial stage on beans which it attacks causing root and collar rot and producing abundant sclerotia (Robertson and Bull, 1956). The fungus also causes the root rot in jute seedlings, coffee, cacao, rubber, banana, citrus, borassus palms, charcoal rot of sweet potatoes (Robertson and Bull, 1956), charcoal stem rot of sunflower in Hungary (Bekesi et al., 1970). Although R. lamellifera is better known as a root parasite, it has been shown to be capable of attacking stems and leaves (Robertson and Bull, 1956).

In diseased oil palm seedlings, the vascular strands which remain after the cortical tissue has been destroyed and the inner surface of the hypodermis are often covered with numerous, minute, subspherical, brownish-black sclerotia which are sometimes visible. These sclerotia have been observed in the roots of diseased oil palm seedlings in different nurseries in Nigeria

(Robertson, 1959a) and have also been reported from Malaysia (Turner and Bull, 1967). Isolations from the sclerotia consistently yielded R. lamellifera which was also isolated from cortical and vascular tissues in an advanced state of decay (Robertson, 1959a and 1959b).

The morphological characteristics of R. lamellifera have been reported by Hopkins (1933) and Robertson (1959a and 1959b). The fungus grew rapidly on artificial media producing a hyaline, appressed mycelium which turned olive green and eventually black as numerous sclerotia measuring between 0·75 mm and 1 mm were produced (Robertson, 1959b). Hopkins (1933) recognised 3 types of hyphae in old cultures. The first type were very fine, hyaline freely-branched hyphae forming a substratum. The second type were brown, consisting of thick-walled narrow or barrel-shaped cells whilst the third type of hyphae were dark-brown, thick-walled and made up of rectangular-shaped cells from which sclerotia were formed.

In physiological studies, Robertson (1959a) found that R. lamellifera grew best at 29°C and 10% soil moisture content. The linear growth rate in corn-meal agar (difco) was between 15 mm and 40 mm a day at 25°C (Radha and Menon, 1957). The fungus liquefied gelatin and its growth appeared to be inhibited by highly concentrated starch media (Hopkins, 1933).

Free mycelial growth in Rhizoctonia bataticola (Taub.) Butler in soil has been reported by Kovoor (Radha and Menon, 1957) who further noted that older

hyphae were destroyed by bacteria at high soil moisture while the younger hyphae continued to grow, colonise vegetable debris and to produce sclerotia for dispersal. Zachariah in 1949 (cited by Radha and Menon, 1957) reported the colonisation of cotton debris by R. bataticola. Radha and Menon (1957) using R. bataticola associated with the coconut wilt disease, however, found that the fungus made free mycelial growth only in sterile soil and was "shy" of soil microflora. Norton's (1953) observation on Sclerotium bataticola Taub. was similar to that of Radha and Menon (1957).

Survival of R. bataticola in natural soil was not affected by different soil moisture regimes but soil moisture was indirectly related to the activity of the fungus by virtue of its influence on the general soil micro-flora (Radha and Menon, 1957).

Robertson (1959b) reported that R. lamellifera was a fungal parasite of Pythium sp. and that this relationship was an important factor in the destruction of cortical tissues and the development of the blast disease in oil palm because it afforded R. lamellifera entry into the host tissues. Thus oil palm seedlings which were initially infected by Pythium sp. appeared to be predisposed to infection by R. lamellifera. Predisposition of host plants to infection by R. lamellifera has also been observed by other workers. Bekesi et al. (1970) reported that seed development in sunflower caused a stress (presumably due to the mobilisation of photosynthetic products from other

parts of the plant to the seeds for storage) predisposing sunflower to infection by Macrophomina phaseoli (Maubl.) Ashby in a similar manner to that suggested by Willie and Calvert (1969) for soybeans.

5.0 Control of the oil palm blast disease

Because the blast disease is primarily a root disease caused by soil-borne fungi, the control measures so far adopted were generally aimed at modifying soil conditions in such a way as to depress the growth and development of the causal organisms. Attempts have been made by various workers to control the disease by cultural and chemical methods and by the selection of resistant varieties of the oil palm.

5.1 Control of the oil palm blast disease by cultural practices

In West Africa and Malaysia where some measures have been adopted for the control of the blast disease of oil palm seedlings, such measures have been based principally on cultural practices. Robertson (1959a) and Gunn et al. (1961), working at the Nigerian Institute for Oil Palm Research (NIFOR), near Benin City, have reported that seedlings planted into the nursery in August and September were more susceptible to the blast disease than the seedlings planted in October. They attributed this to disease escape. Gunn et al. (1961) also observed that seedlings which were planted into the nursery early in the rainy season had a greater benefit of sunshine and adequate soil moisture than seedlings which were planted later. They further found that the heaviest blast attack in the nursery occurred on seedlings which were

small in August and September. On the basis of these findings, Gunn et al. (1961) recommended that oil palm seedlings should be planted into the nursery much earlier than August or September to minimise losses due to the disease. The observation that the date of planting has a profound effect on the incidence of the blast disease was confirmed by Robertson (1959a). Farmers are, therefore, usually advised to plant their oil palm seedlings into the nursery in April or early May (Robertson et al., 1968; Anon, 1965e) because such seedlings were subsequently less susceptible to the blast disease. A similar observation had earlier been made in the Ivory Coast by Bachy in 1957, cited by Robertson (1959a).

Allen (1954) described the effect of various cultural treatments on total loss of seedlings in the nursery at the NIFOR Main Station. Unfortunately he did not make any distinction between seedling losses due to the blast disease and those due to other causes. He found that watering and shading in the dry season reduced losses or increased the size and vigour of oil palm seedlings while mulching, deep hoeing, application of potash and organic manure did not reduce losses.

Observations on the Continuous Nursery Experiment at the NIFOR Sub-Station, Abak indicated that mulching and the incorporation of bunch refuse with soil significantly increased the incidence of the blast disease (Anon, 1957). Mulching with black polythene, which increased soil temperature was observed

to increase oil palm blast incidence in Sabah (Turner and Bull, 1967). Bachy (1958) showed that when oil palm seedlings were shaded from October to February, the incidence of the blast disease was considerably reduced. Similar results were obtained at the NIFOR Main Station (Anon., 1965a; Anon., 1968a and Anon, 1969), Ogba Farm and Abak (Gunn et al., 1961).

Experimental results at the NIFOR Main Station on the effect of watering or irrigation on the blast disease are conflicting. The earliest experiments showed that overhead irrigation particularly in August and September significantly reduced the incidence of the disease (Anon., 1960; Anon., 1961). Hand watering,though beneficial,was less effective than irrigation. Later experiments confirmed these results and showed further that irrigation was less effective than shading in the control of the blast disease (Anon., 1968a). More recent experiments, however, have indicated that irrigation increased the incidence of the oil palm blast disease (Anon., 1967a; Anon., 1969).

The incorporation of organic manure with soil was found to increase the incidence of the blast disease but the slightly higher losses resulting from such organic manuring were shown to be largely offset by the greatly improved growth of surviving seedlings giving a higher percentage of transplantable seedlings (Gunn et al., 1961).

Application of potassium to oil palm seedlings in the nursery increased the number and size of transplantable palms but also significantly increased the

incidence of the blast disease (Forde et al., 1968). Application of sulphate of ammonia to nursery soil at the NIFOR Sub-Station, Abak was found to increase the incidence of blast while application of lime appeared to decrease the number of affected seedlings (Anon, 1957).

The incorporation of organic and inorganic manure with soil together with deep ploughing, the planting of oil palm seedlings into nurseries in April (the beginning of the rainy season in Nigeria), mulching and watering have become standard practices adopted in Nigerian oil palm nurseries. These practices appear to increase the vigour of the seedlings thereby increasing their resistance to the blast disease. Despite the adoption of these cultural practices, however, oil palm seedling losses in Nigeria due to the blast disease are generally high; often exceeding 8%. For example, the losses due to the blast disease among extension work seedlings at the NIFOR Main Station were 8.9% in 1970 (Anon., 1970a), 13.8% in 1971 (Anon., 1971a) and 9.0% in 1972 (Anon., 1972). It was, therefore, considered necessary to find more effective methods of control of the disease.

5.2 Chemical control of the oil palm blast disease.

The earliest experiments at the NIFOR Main Station on the chemical control of blast were based on the wrong assumption that the disease was caused by nematodes. Soil fumigation with D-D mixture, carbon disulphide and ethylene dichloride dissolved in carbon tetrachloride were applied but

they failed to control the disease. The percentages of dead seedlings in plots fumigated with D-D and carbon disulphide were, on the contrary, higher than in control plots (Anon., 1953).

In a similar experiment, nemagon was shown to have no effect on subsequent incidence of blast (Anon., 1963) Further attempts to control the oil palm blast disease by soil fumigation with "Fosferno 25" containing 25% parathion (Anon., 1954), chloropicrin, carbon disulphide (Anon., 1963) were similarly unsuccessful. The incidence of the disease in plots treated with parathion and chloropicrin was surprisingly higher than in control plots and the situation may have been aggravated by phytotoxicity of the chemicals.

Having determined the cause of the blast disease, Robertson (1959a) then attempted in two experiments to control the disease in nursery beds at the NIFOR Main Station by using various fungicides. In his first experiment, formalin, santobrite and karathane were applied to soil but none of them significantly reduced the incidence of the disease. In the second experiment, botrilex, aretan and dithane M22 were applied in solution to the soil about four weeks prior to the time of the year during which the disease was usually observed. These treatments similarly did not significantly control the blast disease (Robertson, 1959a).

The only apparently successful experiment at the WAIFOR Main Station in which the incidence of the disease was significantly less in plots treated

with Santobrite than in untreated plots was reported in 1959 (Anon., 1959).

Later experiments involving soil treatment with chemicals showed that dithane A40 (dithiocarbamate), botrilex (PCNB), agrosan 5W and aretan 6 (Anon, 1966), dithane A40, vapam, PCNB and captan (Anon., 1957a) and dowfume MC2 - 98% methyl bromide and 2% chloropicrin (Anon., 1968a) failed to control the blast disease.

More recently, treatment of nursery soil with dexon 70DP, terraclor super X, benlate and tillex was found to increase the incidence of the blast disease rather than control it because untreated (control) plots had less blast than the plots treated with these chemicals (Anon, 1971a).

Some chemicals have been used in Nigeria for the control of certain plant diseases, for example, perenox and bordeaux mixture for the control of Phytophthora palmivora (Butl.) Butl. on cocoa (Theobroma cacao L.) (Anon., 1962b) and dithane M45 and captan for the control of Cercospora elaeidis Stey. on oil palm (Elaeis guineensis Jacq.) (Anon., 1968a). No worthwhile result has so far been obtained by chemical control of the oil palm blast disease.

5.3 Control of the blast disease by the selection of resistant varieties of the oil palm.

Attempts have also been made to control the blast disease by the breeding of resistant progenies. Part of the work that has been done at NIFOR on the genetics of the blast disease has shown that the disease is controlled primarily

by dominant genes for resistance (Obasola, 1972). This suggests that only one line needs to be resistant to lower the susceptibility that may be present on the other line. Extensive surveys carried out at NIFOR have also shown that resistance to the blast disease can be inherited and a programme was started to select blast-resistant progenies for the production of NIFOR extension work seeds (Robertson et al., 1968). There are indications also that progenies selected from the Nigerian Rain Forest Zone are less susceptible to the blast disease than those from drier areas of Nigeria (Blaak, 1969).

The observation that certain progenies are more resistant than others was confirmed by the results obtained at the NIFOR Main Station from the planting of some materials obtained from Jamaica and Nigeria. Losses among Jamaican progenies were of the order of 80% compared with an average loss of only 6-7% among first grade extension work seedlings developed in Nigeria (Anon, 1962a). It has also been reported that susceptibility to the blast disease was particularly high in Deli, Jamaica and Angola introductions (Anon., 1963).

Blaak (1969) predicted that oil palm seedling losses due to the blast disease could be reduced from around 50% to 1-6% in progenies obtained by crossing susceptible with resistant varieties.

Unfortunately, the extension work seeds produced at NIFOR are usually not screened for resistance to the blast disease. This should be done to

ensure that only seeds which are found to be resistant to the blast disease are distributed to farmers.

SECTION IV

MATERIALS AND METHODS

1.0 Oil palm seedlings

The seedlings used in this investigation were raised from germinated seeds. The best seeds were extension work seeds (E.W.S.) produced at NIFOR by the controlled pollination of selected dura variety with pollen from pisifera variety of the oil palm. The hybrid seeds so produced gave rise, on germination, to tenera variety of the oil palm (Anon., 1965b).

The seeds were germinated by the dry heat treatment method (Anon., 1965c). The method involved soaking the seeds for 7 days with a daily change of water. The seeds were then spread out in a single layer to dry for about 24 hours under a shade. After drying, the seeds were placed in transparent polythene bags, secured with rubber bands and supported in wooden boxes (12 x 8 x 4 inches). The seeds were heated in a germinator maintained at 39°C for 80 days after which they were soaked again for 7 days with a daily change of water. They were spread out in a single layer to dry again for about 2 hours under shade. The seeds were returned to the polythene bags, kept at ambient temperature (25° - 30°C) and examined twice-weekly to remove sprouted seeds.

The sprouted seeds obtained in this way were too delicate to be planted directly into ground beds in the nursery. They were, therefore, planted

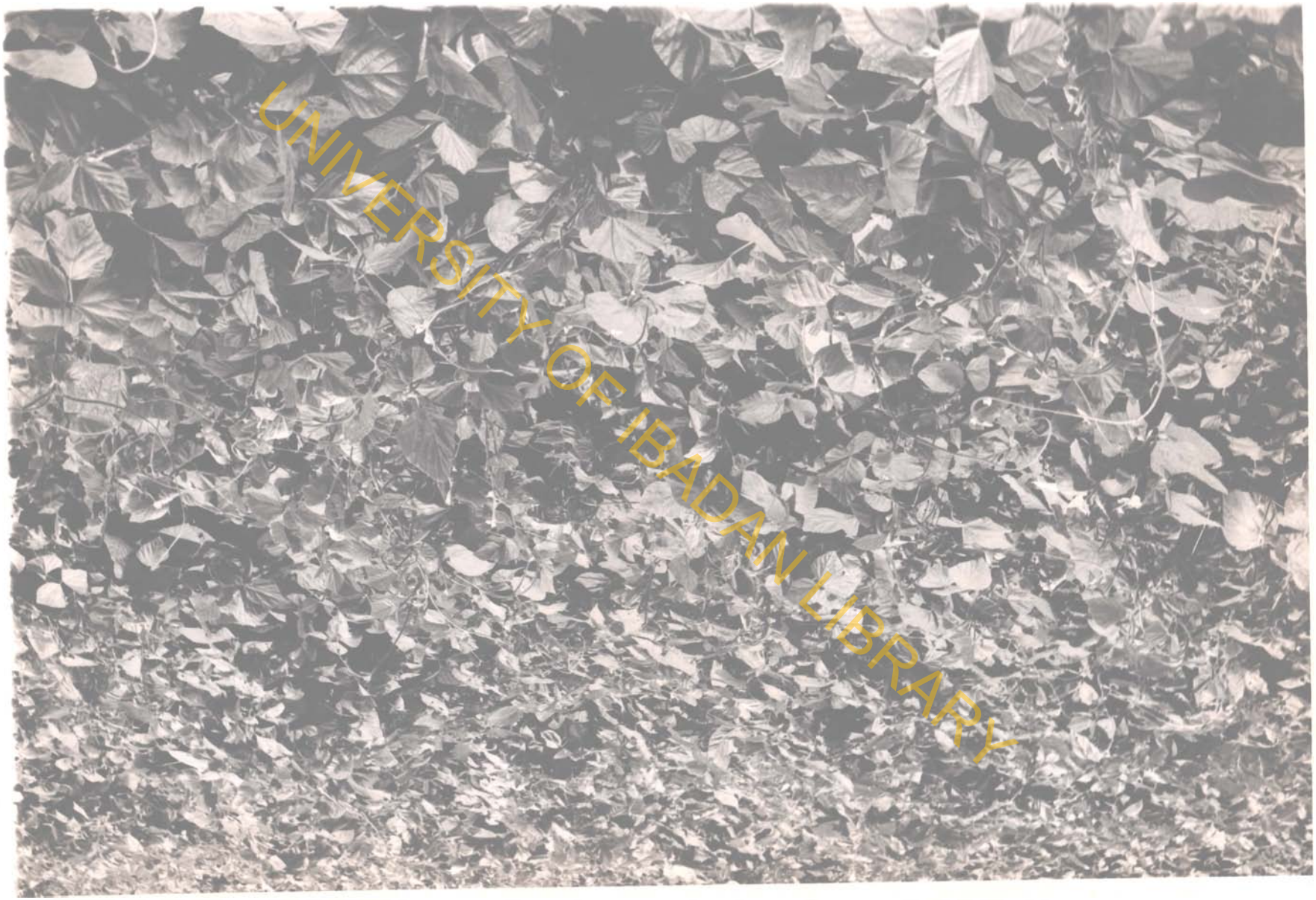
3 x 3 inches apart with their plumules the right way up at a depth of 1 inch in topsoil filled into concrete trays (45 x 21 x 6 inches). The topsoil had previously been partially sterilised by baking on a simple steel framework over an open fire to destroy weed seeds and pests and leaving it to cool afterwards. The trays were then lightly mulched with finely divided bunch refuse, watered by means of overhead spray system and kept free of weeds. A weak solution (about 0.3%) of fertilizers (ammonium phosphate or sulphate of ammonia) was applied to enhance the growth of the seedlings.

Leaf diseases particularly freckle and anthracnose were controlled by spraying the seedlings weekly with a dilute solution of dithane M45. This stage is known as the "prenursery" in the raising of oil palm seedlings and it lasts 4-5 months (Anon., 1965d).

From the prenursery stage, the seedlings were transplanted into the nursery (Anon., 1965e). The nursery land at NIFOR was divided into blocks. When a block had been used for one year it was left under a planted Pueraria fallow for 4-5 years (Plate 3).

Prior to planting seedlings into the nursery, the Pueraria cover and 50 tons of farmyard manure per acre were ploughed into the soil to a depth of 8-10 inches in March of every year. Oil palm seedlings from the prenursery were transplanted into a normal nursery otherwise known as a wet season nursery in April of every year. Seedlings were carefully removed

Plate 3. Fallow nursery site under a planted Pueraria phaseoloides cover crop at the NIFOR Main Station .



from the prenursery with balls of earth around their roots and were planted at $2\frac{1}{2}$ x $2\frac{1}{2}$ feet spacing into either ground beds (ground bed nursery - Plate 4) or polythene bags filled with topsoil from the nursery (polythene bag nursery - Plate 5). The seedlings were mulched with oil palm bunch refuse and were irrigated to 2" of rain per week. Oil palm seedlings in the wet season nurseries were not shaded unless otherwise stated. Animal pests such as slugs, crickets and insect larvae were removed daily from the seedlings. Leaf diseases such as freckle and anthracnose were controlled by spraying the seedlings fortnightly with dithane M45. Weeds were pulled out regularly. Compound fertiliser made up of sulphate of ammonia, superphosphate, sulphate of potash and magnesium sulphate in the ratio of 1:1:1:2 was applied in a ring some 2-3 inches away from the base of the seedlings 3 times during the nursery stage ($\frac{1}{2}$ oz. per seedling in May, $1\frac{1}{2}$ ozs. in July and 2 ozs. in October).

Ground bed nurseries have hitherto been the standard method of raising oil palm seedlings for field planting in Nigeria. Most of the experiments in this investigation were, therefore, carried out in ground bed nurseries. There are indications, however, that ground bed nurseries may soon be replaced in Nigeria by polythene bag nurseries which facilitate the distribution of seedlings to farmers and other planters. For this reason, some experiments particularly on the control of the blast disease were carried out

Plate 4. Oil palm seedlings (7 months old) in a ground bed nursery at the NIFOR Main Station (E).

Plate 5. Oil palm seedlings in a polythene bag nursery at the NIFOR Main Station (F).

E

F

in polythene bag nurseries.

2.0 Survey of the oil palm blast disease

Surveys of blast were carried out at the NIFOR Main Station and in some parts of both the Western and Midwestern States of Nigeria. The oil palm nurseries surveyed for the incidence of the disease are indicated in Fig. 1. In all these places, the disease was identified by reference to the descriptions given by Bull (1954), Robertson and Bull (1956), Robertson (1959a, 1959b) and Robertson et al. (1968) as summarised earlier (Section II).

At the NIFOR Main Station, surveys of the disease were carried out during the blast season (October to March) of every year. The number of seedlings affected by the disease was recorded monthly and such seedlings were pulled up and discarded during each survey to avoid confusion with new cases of infection. Seedling infection due to the blast disease was expressed as a percentage of the total original number of seedlings planted.

For statistical analysis, the percentage blast was transformed (to avoid such figures as zero) by using the formula $Y = \sqrt{x + 0.5}$ derived by Steel and Torrie (1960). In this formula, x denotes the percentage blast, 0.5 is a constant and Y represents the square root of the sum of the percentage blast and the constant (0.5). At the NIFOR Main Station, it was possible to determine the percentage incidence of the disease because this work was carried out in the Institute. In nurseries outside NIFOR, however, it was not

possible to obtain reliable figures on which calculations of percentage infection could be based. Assessment of the blast disease in those areas merely indicated the presence or absence of the disease.

3.0 Culture media, vessels and glassware

Experimental and stock cultures of P. splendens and Rhizoctonia lamellifera were grown in cassava-dextrose agar. In some experiments, Czapek dox -, quaker oats -, potato dextrose -, corn meal -, soluble starch -, V8 juice -, distilled water - and malt extract agar were also used.

All the agar media were first heated in a water-bath to dissolve the agar. Later, 200 ml lots of the media were dispensed into 500 ml - capacity Erlenmeyer conical flasks and then sterilised by autoclaving for 20 minutes at 15 pounds per square inch pressure (15 p.s.i.). The agar media were left to cool to about 45°C before pouring into sterilised petri dishes.

When sterile distilled water was used, the distilled water was sterilised in the same way as the culture media.

Topsoil (the upper 6 inches layer of soil) was sterilised by autoclaving for 1 hour at 15 p.s.i. Petri dishes (9 cm-diameter) and pipettes were sterilised in a hot air oven for 6 hours at 160°C. Cork-borers and inoculating needles were heated until red-hot over the flame of a Bunsen burner.

Whatman's filter paper was placed in a petri dish and sterilised by autoclaving for 20 minutes at 15 p.s.i.

Before using the inoculating room ($6\frac{1}{2}$ x 5 x $10\frac{1}{2}$ feet), it was sterilised by switching on an ultra-violet lamp fitted to a wall for 1 hour.

The following media used in the experiments were prepared as indicated:

Cassava-dextrose agar (CDA)

Cassava flour	135 g
Glucose (D-)	17 g
Difco bacto agar	15 g
Distilled water to	1 litre

Cassava (Manihot utilissima Pohl.) flour was prepared by cutting peeled cassava tubers into thin slices and drying them in the sun. The flakes were ground either in a mortar or in a grinding mill No. A 548.

Cassava-dextrose agar (CDA) was prepared by first soaking 135 g of cassava flour in 500 ml of distilled water in a litre-capacity beaker and heating the suspension in a water-bath at 60°C for 15 minutes with frequent stirring to prevent coagulation. The suspension was filtered through a single layer of Whatman's filter paper No. 44 and 120 ml of the filtrate were made up to a litre with distilled water before glucose and agar were added to the medium.

Corn-meal agar (CMA)

Corn-meal agar (Oxoid CM 103)	17 g
Distilled water	1 litre

Czapek-dox agar (CzDA)

Czapek-dox agar (Oxoid CM 97)	33.4g
Distilled water	1 litre

Distilled-water agar (DWA)

Difco bacto agar	15g
Distilled water	1 litre

Malt-extract agar (MEA)

Difco bacto malt extract	15g
Difco bacto agar	20g
Distilled water	1 litre

Potato-dextrose agar (PDA)

Potato-dextrose agar (Oxoid CM 139)	39g
Distilled water	1 litre

Quaker-oats agar (QOA)

Quaker oats	40g
Difco bacto agar	20g
Distilled water to	1 litre

Quaker oats (compressed white oats-Graan Production N.V. Rotterdam, Holland) were suspended in distilled water, heated in a water-bath at 60°C for 30 minutes and filtered through a muslin cloth. The filtrate was made up to a litre with distilled water and difco bacto agar then added to it.

Soluble-starch agar (SSA)

Soluble starch	40g
Difco bacto agar	15g
Distilled water	1 litre

Sucrose beta-sitosterol agar (SBSA)

Sucrose	2·500g
Asparagine	0·162g
$CaC1_2$	0·052g
$MgS0_4 \cdot 7H_20$	0·100g
KH_2PO_4	0·150g
K_2HP0_4	0·150g
* Trace elements	10 ml
Thiamine HC1	0·001g
Ascorbic acid	0·010g
Beta-sitosterol	0.010g (dissolved in 5 ml of ether)
Difco bacto agar	15·000g
Distilled water to	1 litre

*Trace elements

$ZnSO_4$	0·440g
$FeSO_4$	0·100g
$MnC1_2 \cdot 4H_20$	0·070g

$CuSO_4$	0·008g
NH_4MoO_4	0.005g
Distilled water	1 litre

V8-juice agar (V8JA)

V8 Juice (Campbell soups)	75 ml
Difco bacto agar	20g
Distilled water to	1 litre

Sand culture

Sand (river)	190g
Corn (milled)	10g
Distilled water	20 ml

4·0 Isolation of fungi from infected roots and soils

When it was desired to isolate fungi from infected roots of oil palm seedlings, such roots were washed in running tap water, cut into 0·5 cm-long pieces each of which was split lengthwise into two segments. The segments were rinsed in 3 changes of 1% solution of dihydrostreptomycin sulphate (obtained from Nutritional Biochemicals Corporation, U.S.A.) to inhibit bacterial growth, surface-dried with sterile filter paper and plated on common laboratory agar media such as potato-dextrose agar, corn-meal agar, Czapek-dox agar and malt-extract agar. The plates were incubated at 24-27°C. Fungal colonies growing from the root segments 1-3 days after

plating were subcultured on fresh plates of potato-dextrose agar for identification.

It was, however, necessary to develop a special technique for isolating Pythium spp. from infected roots. Root segments which were obtained, as already described, were rinsed in 5 changes of 1% solution of dihydrostreptomycin sulphate. They were surface-dried with sterile filter paper and plated (5 per plate) on cassava-dextrose agar with the longitudinal section on the agar medium. The plates were incubated at 24-27°C and fungal colonies growing from the root segments after 18-24 hours of incubation were subcultured on fresh plates of cassava-dextrose agar for identification. Colonies of Pythium sp. were identified by reference to the descriptions given by Waterhouse (1967 and 1968).

Sclerotia were found on the stele and inner surface of the hypodermal layer of infected roots of oil palm seedlings. When it was desired to isolate fungi from these sclerotia, they were soaked in 1% solution of dihydrostreptomycin sulphate for 10 minutes, surface-dried on sterile filter paper and plated (5-7 sclerotia per plate) on potato-dextrose agar. Colonies of fungi growing from the sclerotia were subcultured after 24-48 hours of incubation on fresh plates of potato-dextrose agar for identification.

In preliminary experiments to isolate P. splendens from soil, the commonly used soil-plate method first used by Warcup (1950) and the soil-

dilution technique gave unsatisfactory results. The most suitable method of isolating P. splendens from soil was a modification of the baiting technique used by Hine and Luna (1963).

When it was desired to isolate P. splendens from soil, young, live oil palm roots were used as baits. The roots were washed in tap water, cut into 0.1-0.2 cm-thick discs and soaked in 100 p.p.m. of pimaricin (obtained from Royal Netherlands Fermentation Industries) suspension for 1 hour. Twenty root discs were then buried in about 80 gm of the soil sample in a sterile petri dish,. incubated at 24-27°C for 24 hours after which they were recovered, washed again in tap water and rinsed in 5 changes of 1% solution of dihydrostriptomycin sulphate. The root discs were surface-dried with sterile filter paper, plated (5 per plate) on cassava-dextrose agar and incubated at 24-27°C. Fungi growing from the root discs were subcultured after 18-24 hours of incubation on fresh plates of cassava-dextrose agar for identification. Colonies of Pythium species were identified by reference to the descriptions given by Waterhouse (1967 and 1968). Pythium isolation based on the number of root discs colonised by the fungus was then determined as a percentage of the original number of root discs plated.

4.1 Soil sampling for the isolation of fungi

Soil samples used in this investigation were collected from the NIFOR wet season nurseries unless otherwise stated. Samples were taken by

pushing a 4·2 cm internal diameter tubular soil sampler vertically into the soil to a depth of 1 foot. Only plugs of soil from the 9-12 inches zone were collected unless stated otherwise. Ten such plugs of soil, sampled at random, from a nursery were bulked to form a composite sample. Composite soil samples were collected thrice a month from May 1970 to April 1971.

Soil sampling and isolation of fungi were, as a rule, carried out on the same day. When, in exceptional cases, the soil samples were not used immediately for the isolation of fungi, they were stored in tightly closed polythene bags in an air-conditioned room at 20°C for not more than 7 days.

4.2 Identification of isolated fungi

Fungal isolates were identified by reference to the descriptions given by Hopkins (1933), Flentje (1956), Barnett (1958), Robertson (1959a and 1959b), Papavizas and Davey (1962), Sneh et al. (1966) and Waterhouse (1967 and 1968). Cultures of the fungi which were found to be pathogenic to oil palm seedlings were sent to the Commonwealth Mycological Institute for the confirmation of identification.

4·3 Determination of pathogenicity of isolated fungi

The most frequently isolated fungi from infected roots were tested, in inoculation experiments, for pathogenicity on oil palm seedlings. Cultures of the fungi which were grown in cassava-dextrose agar were macerated in a MSE homogeniser and made up to a known volume. Clean jam jars were

filled with fungal macerates and the roots of five oil palm seedlings were immersed in the fungal inocula. The roots of the control seedlings were immersed in macerated cassava-dextrose agar without a fungus. The jars containing the seedlings were kept in an air-conditioned room at 20°C (to minimise shock) for 12 hours. The seedlings were then planted one per plastic pot using sterile topsoil. The pots were arranged behind the laboratory, mulched with oil palm bunch spikelets and watered when necessary. Two weeks after inoculation, individual seedlings were rated visually for disease development on the following scale:

Disease rating	Degree of infection
0	0% (no infection - healthy)
1	10% necrosis of total leaf area or root length
2	20% " " " " " " " "
3	30% " " " " " " " "
4	40% " " " " " " " "
5	50% " " " " " " " "
6	60% " " " " " " " "
7	70% " " " " " " " "
8	80% " " " " " " " "
9	90% " " " " " " " "
10	100% (dead)

Fungi were isolated from artificially-inoculated seedlings by plating infected root segments on cassava-dextrose agar and the frequency of isolation based on the number of root segments colonised by the fungus was calculated as a percentage of the total number of root segments plated.

The growth habit and morphology of fungi isolated from artificially-inoculated seedlings were compared with those of the original fungi used for inoculating the seedlings. A fungus which was isolated from the artificially-inoculated oil palm seedlings and was observed to be identical with the original fungus used for inoculating the seedlings was considered to be pathogenic to oil palm seedlings.

4·4 Inoculation

The inocula consisted of discs (0·4 cm in diameter). When mycelial discs were used as inocula, they were cut with No. 1 (0·4 cm - diameter) cork-borer from the edges of 1-2 day-old colonies of P. splendens or 2-4 day-old colonies of R. lamellifera because the rate of growth of P. splendens is faster than that of R. lamellifera. The discs were then inoculated in the centre of the petri dishes with their mycelial surface on the agar.

4·5 Measurement of linear growth on agar plates

To determine linear growth on agar plates, growth in both fungi was measured along two pre-determined lines at right angles to one another with their point of intersection at the centre of the petri dishes. The mean of the

two measurements was recorded for each replicate. The mean of such measurements for three replicates was recorded as the growth of the fungus on a particular medium under the stated experimental conditions.

4.6 Assessment of sporangial and sclerotial production

Sporangial production was evaluated one week after inoculation while sclerotial production was evaluated two weeks after inoculation unless otherwise stated. Assessment in either case was by counting the sporangia or sclerotia in 8 fields (two in each quadrant of the petri dish) in each plate in a standardised manner under a compound microscope (x 9 objective and x 10 ocular). The mean number of sporangia or sclerotia per field was calculated for each replicate. The mean for 3 replicates was taken as an estimation of the sporangial or sclerotial production.

4.7 Determination of sporangial and sclerotial germination

When it was desired to determine sporangial germination, agar media were inoculated with sporangia obtained from culture media or from inoculated paw-paw fruits. When sporangia were obtained from culture media, two plates of 1-3 week-old cultures of P. splendens were macerated in a MSE homogeniser and made up to 100 ml with sterile distilled water. 2 ml of the macerated cultures were pipetted and spread over the surface of agar culture medium in a petri dish. Sporangia obtained in this manner were not free from nutrients contained in the culture medium.

When it was desired to obtain sporangia free from extraneous nutrients, mature but still green pawpaw (Carica papaya L.) fruits were surface-sterilised with 95% ethanol, inoculated with 0·4 cm-diameter agar disc containing hyphae of the fungus and incubated in a damp chamber for four days at 25 - 27°C.

The aerial mycelium produced on the surface of the fruit was carefully scraped with a sterile inoculating needle into 50 ml of sterile distilled water and macerated in a MSE homogeniser. 2 ml of the sporangia-laden macerate were aseptically pipetted and spread over the surface of 10 ml of solidified agar medium in a petri dish.

Germination and number of germ tubes per sporangium were determined by examining a minimum of 100 sporangia per plate under the low power of the microscope and the percentage germination was calculated.

A sporangium was taken as having germinated when the length of any of its germ tubes was equal to, or greater than that of half its diameter.

When it was desired to determine the germination of sclerotia of R. lamellifera, a four-five-week-old culture of the fungus grown in 20 ml of corn-meal agar at 20°C was macerated in 50 ml of distilled water in a MSE homogeniser. The macerate was poured into a litre of tap water in a two-litre-capacity beaker, agitated and the sclerotia allowed to settle. The supernatant liquid was decanted leaving the sclerotia which were washed again with

a litre of tap water. The process was repeated five times and the sclerotia then transferred into a test-tube where they were further washed in five changes of sterile distilled water. The sclerotia were finally suspended in 5 ml of a 0·1% solution of dihydrostreptomycin sulphate to suppress bacterial growth. The suspension of sclerotia was aseptically pipetted and spread over the surface of sterile Whatman's filter paper and five sclerotia picked up with a sterile needle and inoculated equidistantly on 10 ml of solidified agar medium in a petri dish. Germination was determined under the low power of the microscope and the percentage germination calculated.

A sclerotium was taken as having germinated when the length of any of its germ tubes was equal to or greater than that of half its diameter.

5·0 Preparation of relative humidity solutions

In some experiments, it was necessary to store sclerotia of R. lamellifera at various relative humidity values. The different relative humidity values were obtained by preparing concentrated solutions of various salts as described by the Commonwealth Mycological Institute (Anon., 1968c). Relative humidity values of 32%, 73%, 84% and 95% were obtained by using saturated solutions of calcium chloride, equal amounts of ammonium chloride and potassium nitrate, potassium bromide and sodium sulphite respectively. Values of 45% and 100% relative humidity were obtained by using an air-conditioned room (20°C constant temperature) and sterile distilled water respectively.

6.0 Determination of soil moisture

The moisture content of soil samples from which fungi were isolated was determined by the gravimetric method in three replicates. The samples were first weighed and then dried to constant weight in a hot air oven at 100°C, cooled in a desiccator and weighed again. The loss in weight due to moisture loss was calculated as a percentage of the dry weight of soil. The mean value for the three replicates was taken as the moisture content of the soil sample.

7·0 Determination of soil temperature

Thermometers calibrated in degrees fahrenheit were inserted vertically in soil with their bulbs at a depth of 7 inches from the soil surface. Soil temperatures were taken at 1400 hours daily from May 1970 to April 1971 and the mean value for each month calculated.

8·0 Compound fertilisers

When compound fertilisers were used, they were prepared by mixing together sulphate of ammonia, superphosphate, sulphate of potash and calcined magnesium sulphate in the ratio of 1:1:1:2. The major plant nutrient components of the compound fertilisers were nitrogen, phosphorus, potassium and magnesium.

9·0 Fungicides and other organic chemicals

Various chemicals have previously been tested for the control of the

blast disease but none of them has been found to be promising. One of the objectives of this investigation was to evaluate the effectiveness of some newer and readily available chemicals in the control of the disease.

The following chemicals were used in the experiments:

(a) those which have been recommended by chemical manufacturers to be effective against Pythium or Rhizoctonia and

(b) those already available in Nigeria but are being used for the control of other diseases e.g. captan and dithane M45 for the control of Cercospora elaeidis Stey. on the oil palm (Elaeis guineensis Jacq.) (Anon., 1967a) and also weeds e.g. gramoxone.

The names and sources of supply of the chemicals tested in the laboratory are given as follows:

(i) Benlate - 50% methyl-N-benzimidazol-2-yl-N-(butylcarbomoyl) carbamate supplied by E.I Du Pont De Nemours & Company Inc., U.S.A.

(ii) Captan - 50% N-(trichloromethylthio) cyclohex-4-ene-1, 2-dicarboximide supplied by California Spray Chemicals, Paris.

(iii) Dexon - 70% p-dimethylaminobenzenediazo sodium sulfonate supplied by Chemagro Corporation, U.S.A.

(iv) Dithane M45 - 80% Zinc-manganese and ethylene disdithiocarbamate supplied by Lennig Chemicals Limited, England.

(v) Fernasan - 75% tetramethylthiuram disulphide supplied by Plant Protection Limited, England.

(vi) Gramoxone - 1,1-dimethyl-4,4-bipyridylium supplied by Imperial Chemical Industries, Lagos, Nigeria.

(vii) PCNB - 75% pentachloronitrobenzene supplied by Hoechst Ag. Frankfurt.

(viii) Terraclor super x (emulsifiable) - 23.2% pentachloronitrobenzene and 5.8% 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole supplied by Olin Chemicals, U.S.A.

(ix) Terrazole - 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole supplied by Olin Chemicals, U.S.A.

Solutions or suspensions of the chemicals in sterile distilled water were prepared. The weight of the chemical used was calculated to give a definite concentration in parts per million of the active ingredient unless otherwise stated.

When it was desired to impregnate agar media with the chemicals, 1 ml. of the chemical suspension was aseptically pipetted into and mixed thoroughly

with 9 ml of the agar medium just before pouring into plates.

From the results obtained in the laboratory screening of various chemicals, only Fernasan, Benlate and Pentachloronitrobenzene which were found to be promising were selected for nursery trials on the control of the oil palm blast disease.

When it was desired to treat the soil in the polythene bag nursery with fungicides to control the disease, Pentachloronitrobenzene (PCNB), Fernasan and Benlate were used. The weight of soil required to fill a polythene bag (20" x 15" layflat and 0.005" gauge) was determined. The moisture content of the soil was then determined gravimetrically. From these results, the oven dry weight of soil required to fill a polythene bag was calculated.

Experience with watering of oil palm seedlings in the polythene bag nurseries had shown that 2.5 pint-volume of water was adequate to saturate the soil in a polythene bag. When calculated weights of the fungicides were suspended separately in 2.5 pints of water and applied to the soil in a polythene bag, the concentration of PCNB, Fernasan or Benlate was equivalent to 75, 75 or 10 p.p.m.(weight/weight) respectively. The soil in the polythene bag nursery was treated with these fungicides in October, November and December.

10.0 Assessment of the activity of organic chemicals

The efficiency of the fungicides in inhibiting the linear growth in P.

Plate 6. Shading of polythene bag seedlings (6 months old) with pruned oil palm leaves supported by a frame-work of sticks.

splendens and R. lamellifera was assessed in the laboratory. Plates of cassava-dextrose agar impregnated with the chemicals were inoculated with agar disc inocula and incubated at 24-27°C. Control plates of cassava-dextrose agar were not impregnated with fungicides. Linear growth was measured after a known period of incubation. The difference in the linear growth of the fungi in control plates and on agar medium impregnated with the fungicide was expressed as a percentage (percentage inhibition) of the linear growth in control plates.

To assess the effectiveness of the fungicides in inhibiting germination, plates of cassava-dextrose agar impregnated with the fungicides were inoculated with sporangia of P. splendens or sclerotia of R. lamellifera. Control plates were not impregnated with fungicides. Germination was determined as described in section 4·7. Percentage inhibition of germination was calculated based on the germination obtained in control plates.

11·0 Mulching, watering and shading

When oil palm seedlings were planted in ground beds in the nursery, bunch refuse was used for mulching. When the seedlings were planted in polythene bags, bunch spikelets were used for mulching. The mulch was replenished when necessary.

Oil palm seedlings were watered by using cans or "Wright-rain" overhead sprinkler equipment. When watering cans were used, ½ gallon of water

Plate 7 . Polythene bag seedlings (6 months old) under the shade of 15 year-old plantation palms at the NIFOR Main Station . Note the fence of wire netting designed to protect the seedlings against animal pests .

was applied twice weekly to each seedling unless otherwise stated. When irrigated, the seedlings were supplied with water equivalent to 2" of rain per week (equivalent to 1 gallon of water per seedling per week).

In the shade experiments, each plot was shaded on the eastern and western sides and overhead at a height of 7 feet above the ground with pruned oil palm leaves supported by a framework of sticks (Plate 6). The seedlings were shaded only in the dry season (September to February) of every year unless otherwise stated.

In some experiments shade over oil palm seedlings was provided by 15-year-old planted palms (Plate 7) or forest trees (Plate 8). In such experiments, the forest undergrowth of shrubs and weeds was cleared to provide space for planting the oil palm seedlings.

12.0 Field planting of oil palm seedlings

To establish young palms in the field, one-year-old nursery seedlings were transported with balls of earth around their roots to the field and were planted at a recommended spacing of 29 feet triangular in April or May (Anon., 1965f). In one-year-old seedlings from a polythene bag nursery, the polythene bags were torn off and the seedlings planted with balls-of-earth at the usual 29 feet triangular spacing (Plate 9). Some old leaves of the young palms were pruned prior to transportation to the field leaving 6 - 7 fully-opened leaves per palm. The object of pruning some leaves was to minimise

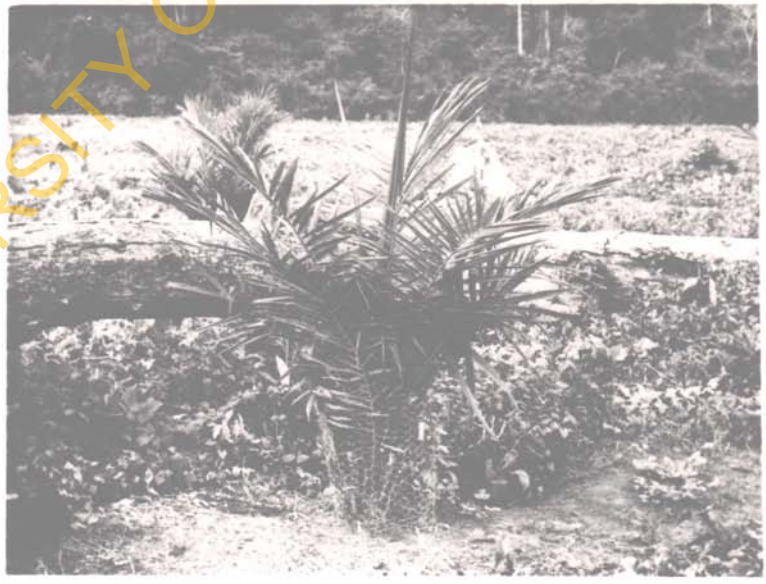
Plate 8. Polythene bag seedlings (6 months old) under the shade of secondary forest vegetation at the NIFOR Main Station. Note the fence of wire netting designed to protect the seedlings against animal pests.

transpiration and shock at planting. Leguminous cover crops were established between the rows of palms by sowing a mixture of Pueraria, Calopogonium and Centrosema (ratio of 2:2:1) in May of the year the oil palm seedlings were planted into the field (Anon., 1967b). Six weeks after planting the young palms into the field, a mixture of $\frac{1}{2}$ lb of ammonium sulphate and $\frac{1}{2}$ lb of potassium sulphate was broadcast in a circle 5-6 feet in diameter around the base of each palm. This was followed by a further application of $\frac{1}{2}$ lb of ammonium sulphate in October of the year of field planting (Anon., 1965g). The young, newly-planted palms were protected from rodents with a collar of wire netting (Plate 10) and were further cared for by regular slashing of the weeds and spraying of the palms with dithane M45 to control leaf diseases (Anon., 1968b).

Plate 9. Young palms in the field (9 months old) at the NIFOR Main Station. Secondary forest can be seen in the back ground (C).

Plate 10. A young palm in the field (9 months old) at the NIFOR Main Station. Note the wire collar designed to protect the young palm against attack by rodents (D).

C



D

SECTION V

EXPERIMENTAL WORK AND RESULTS

1.0 SURVEY OF THE BLAST DISEASE IN SOUTH-WESTERN NIGERIA

Both the leaf and root symptoms of the blast disease have been described by Bull (1954), Robertson and Bull (1956), Robertson (1959a, 1959b) and Robertson et al. (1968) as earlier reported (Section II). These descriptions were used in identifying the disease in oil palm nurseries which were surveyed for the disease in the Western and Midwestern States of Nigeria between 1967 and 1972. The aim of the survey was to record the incidence and extent of the spread of the disease in south-western Nigeria, and the areas under survey are indicated in Fig. 1. The results of the various surveys are given under separate sub-headings.

1·1 Survey of the blast disease at the NIFOR Main Station

Surveys of the blast disease at the NIFOR Main Station were carried out at monthly intervals during the blast season (October to March) of every year from 1967 to 1972. During each survey, infected seedlings were counted, pulled up and discarded to avoid confusion with new cases of infection. The number of seedlings affected by the disease was expressed as a percentage of the total, original number of seedlings planted. The results are given in Table 1.

Fig. 1. Location of oil palm nurseries surveyed for the blast disease in south-western Nigeria.

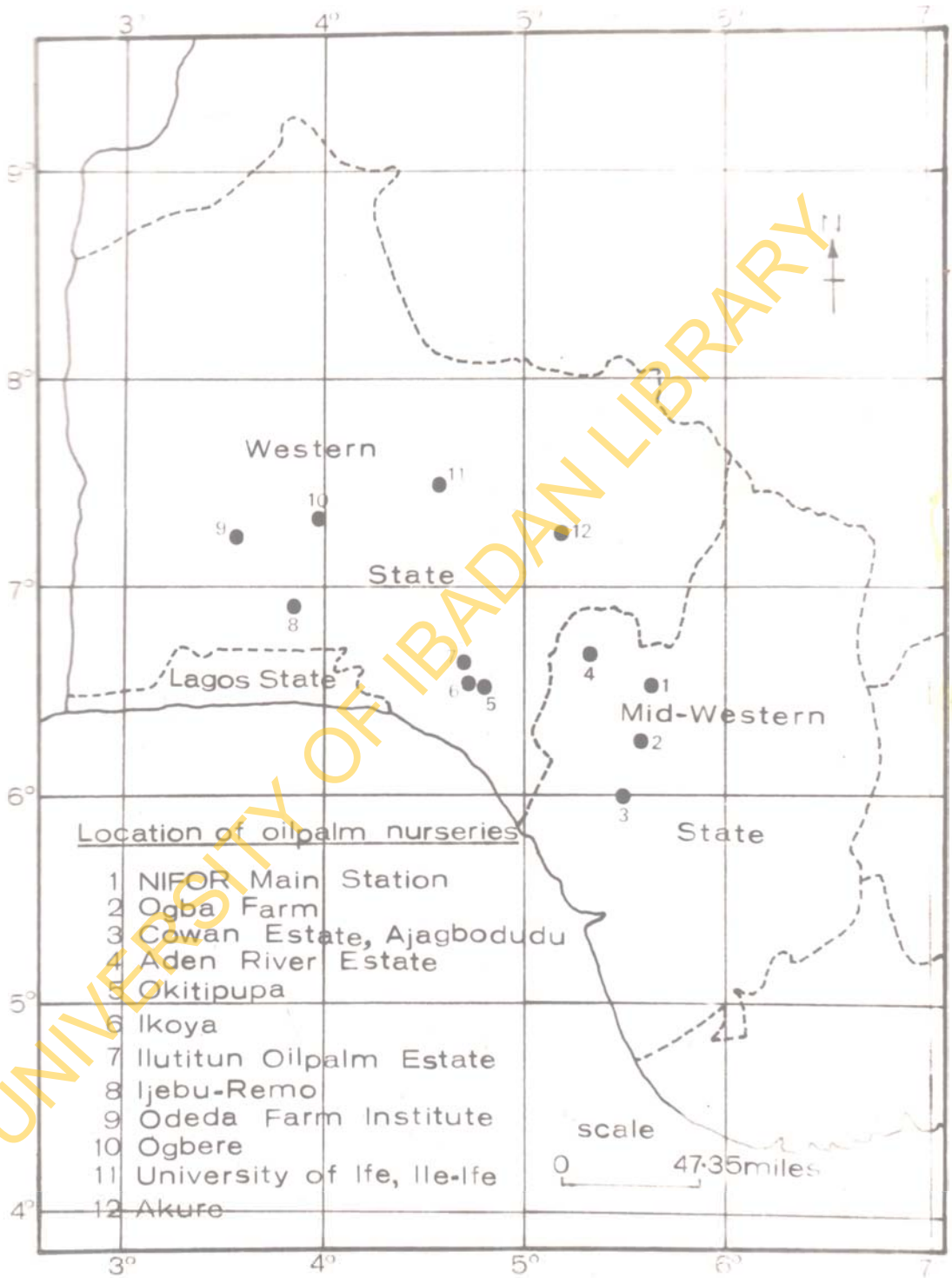


Table 1

Percentage of seedlings affected by the blast disease at the NIFOR Main Station

Months of Survey	Percentage infection by the blast disease					Mean for 5 years
	1967/68	1968/69	1969/70	1970/71	1971/72	
October	1.7	0.0	0.6	0.0	0.6	0.6
November	11.7	2.0	3.8	5.6	4.4	5.5
December	1.7	6.5	6.6	2.6	4.2	4.3
January	1.7	2.5	1.8	0.0	3.0	1.8
February	0.0	1.0	0.8	0.4	0.5	0.5
March	0.0	0.5	0.2	0.0	0.1	0.2
Total	16.8	12.5	13.8	8.6	12.8	12.9

Seedling infection by the blast disease varied between 8.6% and 16.8% annually with an average of 12.9% for the five years. Infection was highest in November and December and lowest in October, February and March.

The variety of the oil palm which was observed to be most susceptible to the blast disease was dura x dura.

1.2 Survey of the blast disease in oil palm nurseries outside the NIFOR Main Station

It was not possible to record the incidence of blast on a monthly basis in oil palm nurseries outside NIFOR because the nurseries were far from the Institute where this investigation were carried out. Hence it was not possible

to obtain information on which calculations of percentage infection could be based. The records in these places, therefore, merely indicated whether the blast disease was observed or not. The location of the nurseries, together with the occurrence of the blast disease, is presented in Table 2.

Table 2

Occurrence of the blast disease in oil palm nurseries outside the NIFOR Main Station

Location of oil palm nursery	State	Blast disease	Year of survey
Cowan Estate, Ajagbodudu	Mid-West	+	1967
Ogba Farm	Mid-West	+	1968-71
Aden River Estates	Mid-West	+	1968-70
Okitipupa	West	+	1967
Ikoya	West	+	1967
Ilutitun Oil Palm Estate	West	+	1968-71
Ogbere Oil Palm Estate	West	+	1967
Ijebu-Remo	West	+	1967
School of Agriculture, Akure	West	+	1967
Central Nursery, Akure	West	+	1967
University of Ife, Ile-Ife	West	+	1967
Odeda Farm Institute	West	+	1967

+ signifies occurrence of the blast disease.

Oil palm blast disease was found in all the nurseries surveyed for the disease in south-wes ern Nigeria.

1.3 Survey of the blast disease among other palms

Palms other than the oil palm were also surveyed for one year only for the blast disease at the NIFOR Main Station. The aim of the survey was to determine whether such palms were infected by the disease or not. Where possible the percentage of infection based on the total number of seedlings planted was also recorded. The results are presented in Table 3.

Table 3

Occurrence and level of incidence of the blast disease among other palms

Name of palm	Occurrence of the blast disease	Percentage infection
Aiphanes acanthophylla (Mart.) Burret	+	74
Areca catechu Linn.	+	100
Areca lynn	+	56
Caryota mitis Lour.	-	0
Chrysalidocarpus lutescens H. Wendl.	+	71
Coccothrinax argentea K. Sch.	-	0
Cocos nucifera Linn.	+	41
Copernicia prunifera Mart.	+	2
Phoenix dactylifera Linn.	-	0
Ptychosperma elegans Blume	+	100
Ptychosperma macarthurii H. Wendl.	+	86
Roystonea regia (H.B.K.) Cook	+	69
Sabal umbraculifera Mart.	-	0

+ denotes occurrence of the blast disease

- denotes absence of the blast disease

The blast disease was observed among all the palms surveyed with the exception of seedlings of Caryota mitis, Coccothrinax argentea, Phoenix dactylifera and Sabal umbraculifera.

The percentage infection was very high in Aiphanes acanthophylla, Areca catechu, Areca lynn, Chrysalidocarpus lutescens, Ptychosperma elegans, Ptychosperma macarthurii and Roystonea regia while it was low in Caryota mitis, Coccothrinax argentea, Copernicia prunifera, Phoenix dactylifera and Sabal umbraculifera. In all the palms that showed infection, there was a higher percentage of infection than in the oil palm itself. The only exception was Copernicia prunifera.

One of the palms, Areca catechu, was surveyed for a second year. The high infection observed in the first year (100%) was maintained in the second year (96%).

1.4 Summary of results on the survey of the blast disease

Surveys of the blast disease were carried out between 1967 and 1972 in nine oil palm nurseries in the Western State and four nurseries in the Midwestern State of Nigeria. The disease occurred in all these nurseries. The highest incidence of the disease was recorded in November and December and the least in March.

The blast disease was also observed among palms other than the oil palm at the NIFOR Main Station, near Benin City in the Midwestern State of Nigeria. In a survey of the disease among such palms, 8 out of 13 types of palms were found to be highly susceptible while the remaining 5 appeared to be resistant to the disease.

2·0 AETIOLOGY OF THE OIL PALM BLAST DISEASE

This section deals with the isolation, identification and proof of pathogenicity of fungi associated with the oil palm blast disease.

2·1 Isolation and identification of fungi associated with the oil palm blast disease at the NIFOR Main Station

In this investigation, all the fungi associated with diseased oil palm seedlings were isolated and identified, although more attention was paid to the isolation of Pythium sp. and Rhizoctoria lamellifera which were earlier reported (Robertson, 1959b) to be the causal organisms of the blast disease.

In preliminary experiments to determine the fungi associated with the blast disease of oil palm seedlings, roots which were freshly pulled out were examined with the naked eye and also with the hand lens for fungi. The roots were later washed and plated as described earlier (Section IV) on corn-meal agar, Czapek-dox agar, distilled-water agar, malt-extract agar, potato-dextrose agar and V8-juice agar. The roots of apparently healthy oil palm seedlings were similarly plated on these agar media. The aim of plating healthy roots on the agar media was to compare the type of fungi associated with healthy and diseased roots of the oil palm. All plates were incubated at 25-27°C. Colonies of fungi growing from the root segments were sub-cultured after 1-3 days of incubation on fresh plates of potato-dextrose agar for identification. Isolated fungi were identified by reference to the descriptions

given by Hopkins (1933), Barnett (1958), Flentje (1956) and Waterhouse (1967 and 1968). The genera of fungi isolated from infected and healthy roots of the oil palm are given in Table 4.

Table 4

Fungi isolated from infected and healthy roots of oil palm seedlings

Genera of fungi	Infected roots	Healthy roots
Aspergillus	+	+
Curvularia	+	+
Fusarium	+	+
Penicillium	+	+
Rhizoctonia	+	-
Rhizopus	+	+
Trichoderma	+	+

+ means isolated

- means not isolated

The fungi isolated from infected roots were similar to those from healthy roots of the oil palm. The only exception was Rhizoctonia sp. which was isolated from infected but not from healthy roots.

Sclerotia were found on the stele and hypodermis of diseased seedlings of the oil palm. The sclerotia were soaked in 1% solution of dihydrostreptomycin sulphate for 10 minutes and plated (5-7 sclerotia per plate) on the

agar media already stated. The plates were incubated at 25-27°C. Colonies of fungi growing from the sclerotia were subcultured on fresh plates of potato-dextrose agar for identification.

Rhizoctonia sp. was consistently isolated from sclerotia plated on the agar culture media.

2·2 Special technique for the isolation of Pythium sp. from infected roots of oil palm seedlings

When the agar culture media already referred to were used, it was not possible to isolate Pythium sp. which Robertson (1959b) had reported to be one of the pathogens of the blast disease. Other media and techniques were, therefore, used for the isolation of Pythium sp.

The media used for this experiment were cassava-dextrose agar (CDA), cassava-dextrose agar impregnated with 100 p.p.m. of pimaricin (CDA + pimaricin), corn-meal agar impregnated with 100 p.p.m. of pentachloronitrobenzene (CMA + PCNB), quaker-oats agar (QOA) and soluble-starch agar (SSA). Segments of infected roots were plated as already described on these agar culture media. The plates were incubated at 25-27°C. Fungi growing from the root segments were subcultured on fresh plates of potato-dextrose agar for identification. Pythium sp. was identified by reference to the descriptions given by Waterhouse (1967 and 1968). Table 5 shows the media on which Pythium sp. grew or did not grow from infected roots.

Table 5

Types of agar medium on which Pythium sp. grew or did not grow from infected roots

Media	Pythium sp.
Cassava-dextrose agar	+
Cassava-dextrose agar + pimaricin	+
Corn-meal agar + PCNB	+
Quaker-oats agar	-
Soluble-starch agar	-

+ means isolated

- means not isolated

Pythium sp. grew from infected roots on three out of the five agar media used. The three types of agar medium were cassava-dextrose agar (CDA), cassava-dextrose agar impregnated with 100 p.p.m. of pimaricin (CDA + pimaricin) and corn-meal agar impregnated with 100 p.p.m. of pentachloro-nitrobanzene (CMA + PCNB).

The three types of agar medium were also compared for sensitivity in isolating Pythium sp. The aim of this was to select only the most suitable medium for the routine isolation of Pythium sp. from infected roots.

Each agar medium was plated with 50 segments of infected roots (5 segments per plate). The plates were incubated at 25-27°C. Preliminary

studies had shown that Pythium sp. grew faster than other fungi in these media and the fungus could be subcultured after 18-24 hours of incubation. Colonies of fungi growing the root segments were, therefore, subcultured after 24 hours of incubation on fresh plates of cassava-dextrose agar for identification. The number of Pythium colonies growing from the root segments was expressed as a percentage isolation based on the total number of root segments plated. The results are shown in Table 6.

Table 6

Percentage isolation of Pythium sp. from infected roots of oil palm seedlings

Media	Pythium isolation
	%
Cassava-dextrose agar	70
Cassava-dextrose agar + Pimaricin	44
Corn-meal agar + PCNB	14

The highest percentage isolation of Pythium sp. was obtained in cassava-dextrose agar. The lowest percentage isolation of the fungus was obtained in corn-meal agar impregnated with 100 p.p.m. of pentachloro-nitrobenzene.

A few colonies of Fusarium sp. and Rhizoctonia sp. also grew from

infected root segments plated on cassava-dextrose agar. Pythium sp., however, grew faster than Fusarium sp. or Rhizoctonia sp. on the agar culture medium. Furthermore, the fungus, unlike Fusarium sp. or Rhizoctonia sp., also produced sporangia within 48 hours in cassava-dextrose agar thereby facilitating both isolation and identification. Cassava-dextrose agar was selected, on the basis of these results, for the routine isolation of Pythium sp. from samples of infected roots of oil palm seedlings.

2·3 Fungi associated with the oil palm blast disease outside the NIFOR Main Station

At the NIFOR Main Station, Pythium sp. and Rhizoctonia sp. were the two fungi consistently isolated from root samples of diseased oil palm seedlings. It was, therefore, desirable to find out whether these two fungi were also associated with the oil palm blast disease in nurseries outside the NIFOR Main Station or not.

Three oil palm nurseries in the Midwestern State and nine nurseries in the Western State of Nigeria which were surveyed for the blast disease have already been listed (Section 1·2). Samples of infected roots were collected from diseased seedlings in these nurseries. Segments of the infected roots were plated on cassava-dextrose agar and incubated at 25-27°C. Colonies of fungi growing from the root segments on the agar medium were subcultured after 24-48 hours of incubation on fresh plates of cassava-dextrose agar for

identification. Pythium sp. and Rhizoctonia sp. were identified by reference to the descriptions given by Waterhouse (1967 and 1968) and Flentje (1956) respectively. Table 7 shows whether the two fungi were found in association with the blast disease in oil palm nurseries outside the NIFOR Main Station or not.

Table 7

Association of Pythium sp. and Rhizoctonia sp. with the blast disease in oil palm nurseries outside the NIFOR Main Station

Location of oil palm nursery	State	Types of fungi isolated.		Year of isolation
		Pythium sp.	Rhizoctonia sp.	
Cowan Estate, Ajagbodudu	Mid-West	+	+	1967
Ogba Farm	Mid-West	+	+	1968-71
Aden River Estates	Mid-West	+	+	1968-70
Okitipupa	West	-	+	1967
Ikoya	West	-	+	1967
Ilutitun Oil Palm Estate	West	+	+	1968-71
Ogbere Oil Palm Nursery	West	+	+	1967
Ijebu-Remo	West	-	+	1967
School of Agriculture, Akure	West	+	+	1967
Central Nursery, Akure	West	+	+	1967
University of Ife, Ile-Ife	West	-	-	1967
Odeda Farm Institute	West	-	+	1967

+ means isolated

- means not isolated

Rhizoctonia sp. was isolated from diseased oil palm seedlings in all the nurseries excepting the nursery located in the University of Ife in the Western State of Nigeria.

Pythium sp. was isolated from diseased root samples collected from Cowan Estate, Ajagbodudu, Ogba Farm and Aden River Estates in the Mid-western State and Ilutitun Oil Palm Estate, Ogbere Oil Palm Nursery, School of Agriculture and Central Nursery, Akure in the Western State of Nigeria. The fungus was not isolated from diseased oil palm seedlings in nurseries sited at Okitipupa, Ikoya, Ijebu-Remo, University of Ife and Odeda Farm Institute in the Western State of Nigeria.

Pythium sp. and Rhizoctonia sp. were isolated from diseased oil palm seedlings in the different localities from which samples were collected outside the NIFOR Main Station. The isolates of either of these two fungi were morphologically and culturally identical even though they were obtained from different localities.

2·4 Pathogenicity tests

Pythium sp. and Rhizoctonia sp. which were consistently isolated from the roots of diseased oil palm seedlings were the two fungi tested for pathogenicity on healthy seedlings of the oil palm.

In preliminary experiments, oil palm seeds which were heated at 39°C for 80 days in a germinator were planted in a mixture (ratio of 9:1) of sterile soil and two-week-old sand culture of Pythium sp. in plastic pots. The aim of heating the seeds was to overcome dormancy. Five of such seeds were planted in each pot and there were four replicates. Twenty other pre-heated seeds were planted in a mixture (ratio of 9:1) of sterile soil and two-week-old sand culture of Rhizoctonia sp. The same number of preheated seeds was planted in a mixture (ratio of 8:1:1) of sterile soil, two-week-old sand culture of Pythium sp. and two-week-old sand culture of Rhizoctonia sp. Preheated seeds which were planted in sterile soil without a fungus served as the control. All the pots were mulched with oil palm bunch spikelets and were arranged outside the laboratory for observation.

Seedlings did not emerge from any of the plastic pots after two months of planting the seeds. It was necessary to wait for two months because of poor seed germination under natural conditions and slow development of sprouted seeds of the oil palm. The seeds were then recovered from soil and the kernels were extracted by cracking the shell. The kernels were cut into two segments, washed in five changes of 1% solution of dihydrostreptomycin sulphate (to inhibit bacterial growth) and were plated on cassava-dextrose agar. The plates were incubated at 25-27°C and examined for Pythium sp. and Rhizoctonia sp. after 1-3 days of incubation.

Neither Pythium sp. nor Rhizoctonia sp. grew in the agar plates. It was, therefore, desirable to find another method of testing the fungi for pathogenicity on seedlings of the oil palm.

Dipping the roots of healthy, prenursery seedlings of the oil palm in the fungal inoculum and planting them in sterile soil was found to give reliable and reproducible results.

Four plates of two-week-old cultures of Pythium sp. grown in cassava-dextrose agar were macerated and made up to a litre with sterile distilled water. Four plates of two-week-old cultures of Rhizoctonia sp. grown in cassava-dextrose agar were similarly prepared. For the control, the same number of plates of cassava-dextrose agar without a fungus was macerated and made up to a litre. Three 700 ml-capacity jam jars were filled separately with 660 ml of Pythium sp., Rhizoctonia sp. and non-inoculated cassava-dextrose agar macerates. A fourth jar of the same capacity was filled with a mixture of 330 ml of Pythium macerate and an equal volume of Rhizoctonia macerate.

Dura x dura variety of oil palm seedlings which was earlier recorded (Section 1·1) to be highly susceptible to the blast disease was used for the inoculation experiment. Five-month-old prenursery seedlings were carefully removed from the concrete trays to avoid root damage and the roots were examined to ensure that they were not infected. The roots of five such

seedlings were immersed in the macerate in each jam jar which was kept at 20°C (to minimise shock) for 12 hours. The seedlings were then planted in sterile topsoil in $5\frac{3}{4}$ inches (rim-diameter) plastic pots at the rate of one seedling per pot. After planting, the pots were arranged under a shade. The plants were rated visually for disease development 2 weeks (a period which in a preliminary experiment had been found to be adequate for disease development) after inoculation with the fungi. The result obtained for the rating of disease development is presented in Table 8.

Table 8

Rating of artificially-inoculated seedlings for disease development

A. Leaf symptoms	Infection rating per seedling					
Treatments	Replicates					
	I	II	III	IV	V	Mean
Pythium sp. alone	8	10	3	10	10	8.2
Rhizoctonia sp. alone	0	0	3	2	1	1.2
Pythium sp. + Rhizoctonia sp.	6	6	5	1	1	4.8
Control	5	3	2	0	0	2.0
B. Root symptoms						
Pythium sp. alone	7	10	8	9	9	8.6
Rhizoctonia sp. alone	3	4	5	6	7	5.0
Pythium sp. + Rhizoctonia sp.	6	7	5	6	5	6.0
Control	5	5	6	7	4	4.8

Infection of both the root and leaf was severest among seedlings inoculated with Pythium sp. alone. It was least for those inoculated with Rhizoctonia sp. alone. The infection by the mixture of Pythium sp. and Rhizoctonia sp. was intermediate between these two. There was no significant difference between seedlings inoculated with Rhizoctonia sp. alone and the control. In all cases, a scoring was obtained for the control. This was attributed to shock.

Segments of infected roots from artificially-inoculated and from the

control seedlings were plated on cassava-dextrose agar and incubated at 25°C for the purpose of isolating Pythium sp. and Rhizoctonia sp. from them. The frequencies of re-isolation of both fungi are shown in Table 9.

Table 9

Frequencies of re-isolation of Pythium sp. and Rhizoctonia sp. from artificially-inoculated oil palm seedlings

Treatments	Number of segments plated	Frequencies of re-isolation of fungi (%)	
		Pythium sp.	Rhizoctonia sp.
Pythium sp. alone	45	60	0
Rhizoctonia sp. alone	26	8	70
Pythium sp. + Rhizoctonia sp.	34	50	32
Control	32	0	0

The frequencies of re-isolation of Pythium sp. and Rhizoctonia sp. from seedlings artificially-inoculated with the respective fungi were high indicating that both fungi infected the roots of healthy oil palm seedlings.

Rhizoctonia sp. was also isolated from sclerotia produced in infected roots of seedlings artificially inoculated with Rhizoctonia sp. alone or with the mixture of Pythium sp. and Rhizoctonia sp.

Pythium sp. was isolated from seedlings inoculated with a pure culture of Rhizoctonia sp. alone. The low frequency of isolation (8%) was attributed to

contaminated pot soil or homogeniser during the maceration of fungal cultures. When this experiment was repeated and more care was taken to avoid contamination of pot soil or homogeniser, Pythium sp. was not isolated from seedlings artificially inoculated with Rhizoctonia sp. alone.

Pythium sp. and Rhizoctonia sp. which were re-isolated from artificially-inoculated seedlings were identical culturally and morphologically with their original cultures. The two fungi were, therefore, considered to be the pathogens of the oil palm blast disease.

Cultures of Pythium sp. and Rhizoctonia sp. which were found in these inoculation experiments to be pathogenic to oil palm seedlings were sent to the Commonwealth Mycological Institute (CMI) for confirmation of identification. The fungi were identified as Pythium splendens Braun (Accession No. IMI 149554) and Rhizoctonia lamellifera Small (Accession No. IMI 149556) by Dr. Stamps and Dr. Mordue respectively. These fungi were used in all subsequent biological and cultural studies in this investigation.

The following observations were made on artifically-inoculated seedlings:

A. Seedlings inoculated with Pythium sp.

The leaves of the seedlings did not show the colour changes from green to olive-green then to greenish-yellow and purplish-brown which are characteristic of diseased seedlings. Loss of gloss was rapidly followed by

leaf necrosis and desiccation. The spear leaves were rotten.

Root symptoms were typical of those of diseased seedlings under natural conditions. Cortical tissues were completely destroyed leaving the vascular strands hanging loosely within a hollow hypodermis. Sclerotia were absent from the roots.

B. Seedlings inoculated with Rhizoctonia sp.

The outer whorl of leaves became flaccid and showed a die-back from the tips. There was no extensive leaf necrosis or desiccation typical of the blast disease. The spear leaves showed neither basal nor apical necrosis.

Infection was restricted to the tips or distal half of the roots with well-defined transition zones. Sclerotia were present in the small, secondary roots.

C. Seedlings inoculated with a mixture of Pythium sp. and Rhizoctonia sp.

The leaves became flaccid and the outer whorls showed colour changes from green to yellowish-green, then to pale yellow and later to brown. Leaf necrosis and desiccation were slower than in seedlings inoculated with Pythium sp. alone. The central spear leaf became necrotic.

Root damage though extensive was less severe than among seedlings inoculated with Pythium sp. alone. There was no marked transition zone.

Sclerotia were found on the stele and hypodermis of both primary and secondary roots.

D. Control seedlings

Two out of the 5 seedlings had healthy-looking leaves. Most of the leaves of the remaining three seedlings became dull and flaccid and showed a die-back from the tips. A few leaves turned brown.

The roots which were inadvertently damaged during the lifting of seedlings from the prenursery became rotten but the roots which were not damaged at that time remained healthy. No sclerotia were found in the rotten roots and Pythium sp. and Rhizoctonia sp. were not isolated from the roots of the seedlings.

2.5 Summary of results on the aetiology of the oil palm blast disease

Pythium splendens Braun (Accession No. IMI 149554) and Rhizoctonia lamellifera Small (Accession No. IMI 149556) were consistently isolated from oil palm seedlings with the blast disease but not from healthy oil palm seedlings at the NIFOR Main Station. The two fungi were also found in association with the oil palm blast disease in nurseries outside the NIFOR Main Station.

R. lamellifera was isolated by plating sclerotia or segments of infected roots on corn-meal agar, Czapek-dox agar, distilled-water agar, malt-extract agar, potato-dextrose agar and V8-juice agar. P. splendens was, however, not isolated when infected roots were plated on these agar media. The most satisfactory method of isolating the fungus was by plating segments of infected roots on cassava-dextrose agar.

P. splendens and R. lamellifera were shown, in pathogenicity tests, to co-infect oil palm seedlings resulting in the blast disease. Under experimental conditions, P. splendens was found to be more pathogenic on oil palm seedlings than R. lamellifera .

3.0 ECOLOGICAL STUDIES ON P. SPLENDENS

In an earlier study relating to the aetiology of the blast disease, P. splendens and R. lamellifera were consistently isolated from infected roots and both fungi were shown in inoculation experiments to be the causal organisms of the oil palm blast disease (Sections 2.0 - 2.4). An ecological study on P. splendens in soil was then made because the fungus was found to be more pathogenic on oil palm seedlings than R. lamellifera under experimental conditions.

3.1 Vertical distribution of P. splendens in soil

In order to investigate the distribution of P. splendens in soil for the purpose of determining the soil depth at which the greatest population of the infective propagules of the fungus could be found, soil samples were taken from the nursery in which oil palm seedlings had been growing at the NIFOR Main Station. The samples were taken from the following soil depths - 1, 3, 6, 9, 12, 18, 24 and 36 inches.

The isolation of P. splendens from soil by the soil-dilution or soil-plate method was found to be unsatisfactory because of the masking effect of fast-growing and heavily-sporulating fungi. The most suitable method of isolating the fungus from soil was a modification of the baiting technique used by Hine and Luna (1963). The modified technique, described earlier (Section IV) was used for isolating P. splendens from the soil samples. The isolation

was repeated with discs (0·4 cm-diameter and 0·1-0·2 cm-thick) of cassava tuber as baits. Pythium isolation percentage was determined on the basis of the number of discs colonised by the fungus. The results are shown in Table 10 and illustrated in Fig. 2.

Table 10

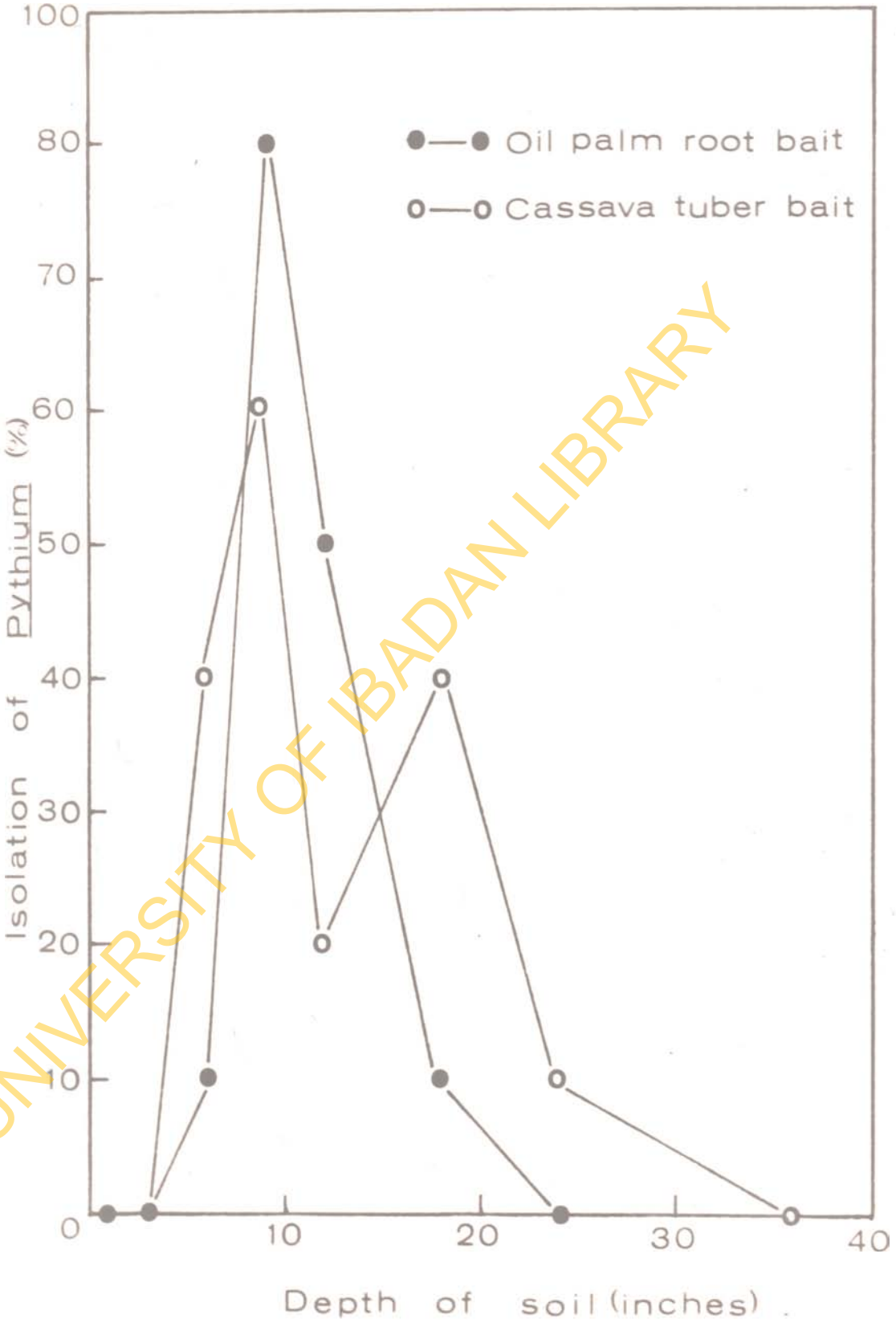
The distribution of P. splendens according to soil depth in nursery at the NIFOR Main Station

Depth of soil (inches)	Pythium isolation percentage	
	Discs of oil palm root	Discs of cassava tuber
1	0	0
3	0	0
6	10	40
9	80	60
12	50	20
18	10	40
24	0	10
36	0	0

The highest recovery of P. splendens was obtained from a soil depth of 9 inches (Fig. 2). The fungus was not isolated from the upper 3 inches layer of soil or from a depth of 36 inches.

This pattern of distribution of the fungus in soil was similar for both types of bait. Discs of live roots of the oil palm were found to be more

Fig. 2. Recovery value of P. splendens from different depths of nursery soil at the NIFOR Main Station.



suitable than discs of cassava tuber for isolating P. splendens from soil. In subsequent experiments on the isolation of P. splendens, soil samples were taken from a zone of 9-12 inches from the soil surface since it was felt that this would give a satisfactory estimate of the organism in soil. Discs of live oil palm roots were used as baits.

3.2 Effect of seasonal variation in soil moisture and soil temperature on the recovery of P. splendens from soil by the root baiting technique

The effect of seasonal changes in the two critical factors commonly found in the tropics - soil moisture and soil temperature-on the recovery of P. splendens was investigated.

A portion of the nursery at the NIFOR Main Station was selected for this study. Oil palm seedlings were planted on the site in April at the beginning of the rainy season in Nigeria. After transplanting the young palms into the field in April of the following year, soil samples were taken from a zone of 9-12 inches below the ground level. Eight such samples were bulked to form a composite sample. Composite samples were taken thrice a month for one year beginning from May. Soil moisture content was determined gravimetrically and the mean value for each month calculated. Soil temperature was determined by means of four thermometers inserted vertically in soil with the bulbs at a depth of 7 inches from the soil surface. Readings were taken at 1400 hours daily and the mean value for each month

was then calculated. The isolation of P. splendens from the soil samples was by the root baiting technique described earlier (Section IV). Pythium isolation percentage was calculated on the basis of the number of root discs colonised by P. splendens. The mean Pythium isolation percentage for each month was calculated from the determinations carried out three times in a month and the value was taken as a measure of the recovery value of the fungus from soil. The results are presented in Table 11 and illustrated in Figs. 3 and 4.

Table 11

Effect of soil moisture and temperature on the recovery value of P. splendens from nursery soil at NIFOR Main Station

Month of determination	Mean Pythium isolation percentage	Mean soil temperature (^{o}F)	Mean soil moisture (%)
May	48.3	85.5	8.6
June	48.3	84.6	8.1
July	63.3	82.3	9.0
August	80.0	81.2	10.3
September	96.7	82.2	10.7
October	83.3	86.2	10.3
November	25.0	91.6	5.2
December	38.3	87.2	3.6
January	38.3	84.6	5.2
February	73.3	88.8	4.8
March	68.3	87.8	5.9
April	76.7	88.9	7.2

Fig. 3. Relationship between seasonal variation in soil moisture and the recovery of P. splendens from oil palm nursery at the NIFOR Main Station.

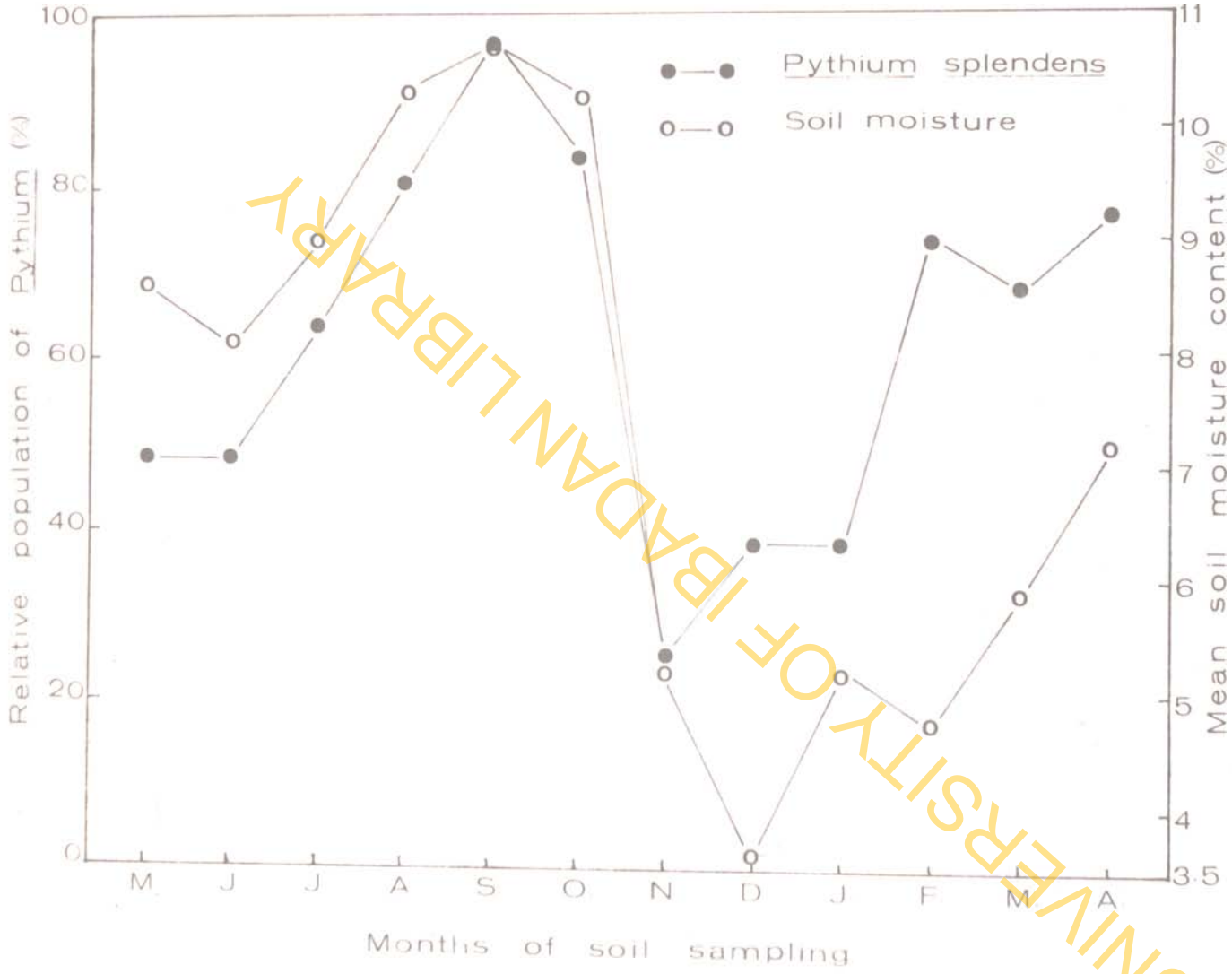
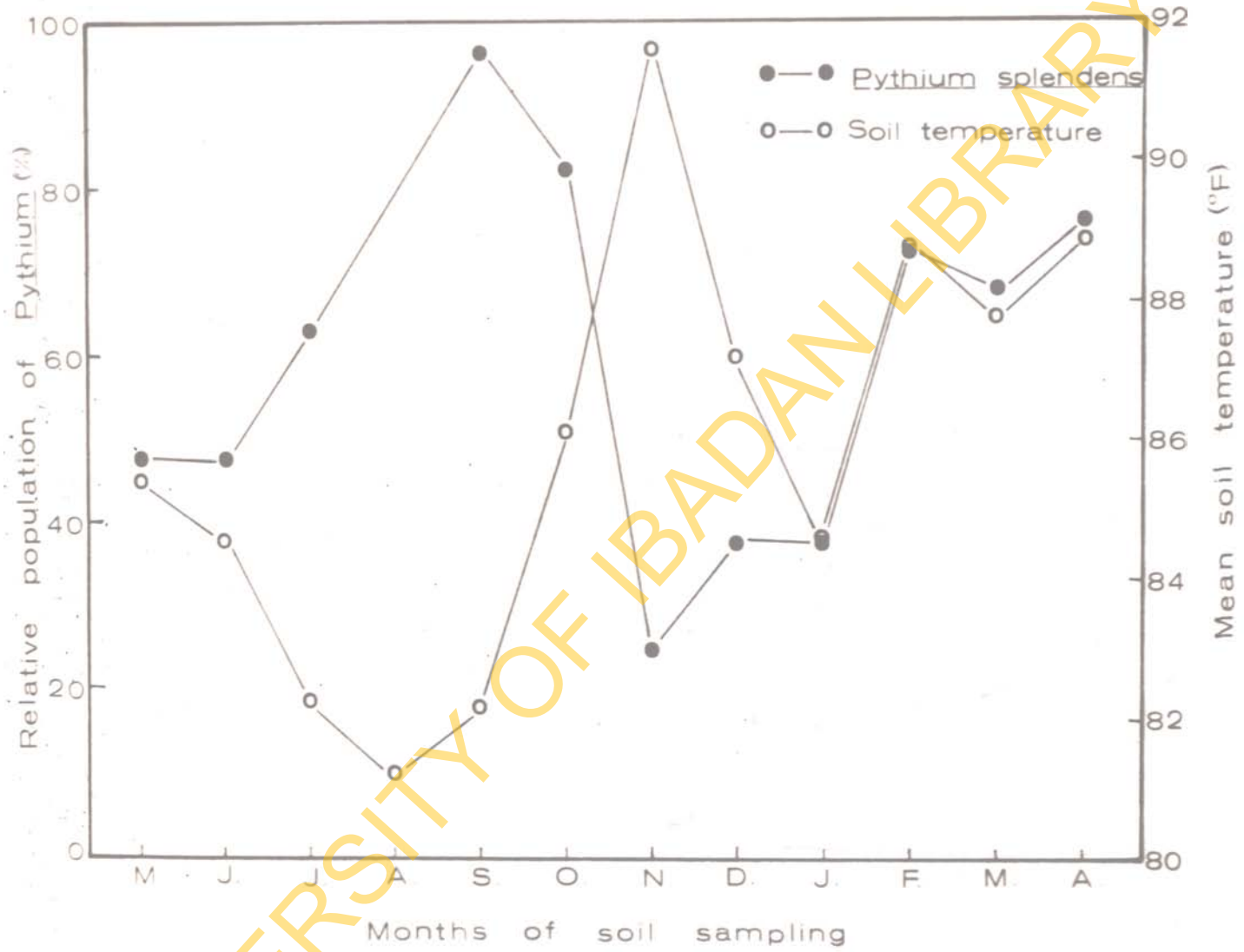


Fig. 4. Relationship between seasonal variation in soil temperature and the recovery of P. splendens from oil palm nursery at the NIFOR Main Station.



In May and June, about 50% recovery of P. splendens was obtained from soil. From July, it increased to more than 60% reaching a maximum of nearly 100% in September. In October, the recovery value was still very high but between November and January, it fell to a low value of between 25-40%. The recovery of the fungus from soil built up again in February-April but it was not as high as in August-October.

During this period, the deviation in soil temperature was not too high. Differences in the recovery of P. splendens from soil were not likely to be affected by changes in soil temperature.

Soil moisture was highest in August-October and this corresponded with the highest recovery value of P. splendens. Thereafter, it fell sharply and this also corresponded with a fall in the recovery of the fungus from nursery soil.

3·3 Effect of air-drying of soil on the viability of P. splendens as determined by the root baiting technique

Soil moisture was earlier found to have a profound effect on the recovery of P. splendens from soil (Section 3·2). The effect of air-drying of soil on the viability of propagules of P. splendens was investigated in this experiment.

Ten soil samples, taken from a wet season nursery at the NIFOR Main Station, were bulked to form a composite sample. The moisture content in the fresh soil sample was determined gravimetrically. The recovery of P.

splendens from the fresh soil sample was determined by the root baiting technique. The composite soil sample was then divided into two parts. One part was placed in the moist state in tightly closed polythene bags and stored at 25-27°C. The other half was spread out in a thin layer on a sheet of cardboard paper to dry for 2 days at 25-27°C. To determine the viability of Pythium propagules, a portion of the soil sample was saturated with distilled water and 50 discs of live oil palm (dura variety) roots were buried in it for 24 hours. The root discs were then plated on cassava-dextrose agar and the number of discs colonised by Pythium was determined after 18-24 hours of incubation. Viability of Pythium was determined on the basis of the number of root discs colonised by the fungus. The results are shown in Table 12.

Table 12

Viability of P. splendens as determined by the root baiting technique in moist and air-dried nursery soil

Air-dried soil		Moist soil	
Soil moisture (%)	Viability of P. splendens (%)	Soil moisture (%)	Viability of P. splendens (%)
8·4	54	8·4	54
1·2	14	8·3	56

When the soil sample was air-dried for two days, the soil moisture content was reduced from 8·4% to 1·2% and there was a corresponding reduction

in the viability of P. splendens as determined by the root baiting technique from 54% to 14% . There was practically no alteration in the moisture content and viability of P. splendens in the moist soil sample .

3·4 Effect of period of storage on the viability of P. splendens as determined by the root baiting technique

The effect of air-drying of soil on the viability of P. splendens has already been investigated (Section 3·3) . Both the air-dried and moist soil samples used in the last experiment (Section 3·3) were stored in tightly closed polythene bags at 25–27°C . Soil moisture content was determined gravimetrically after varying periods of storage . Viability of P. splendens was determined periodically by the root baiting technique . The results are presented in Table 13 .

Table 13

Viability of P. splendens as determined by the root baiting technique after varying periods of storage at 25–27°C

Period of soil storage (days)	Air-dried soil		Moist soil	
	Soil moisture (%)	Viability of P. splendens (%)	Soil moisture (%)	Viability of P. splendens (%)
5	1·3	2	8·3	52
9	1·2	4	8·2	54
16	1·2	0	8·3	54
23	1·3	0	8·1	52

The moisture content of the air-dried soil was about 1.2% and this value did not vary much during the 23-day period of soil storage. There was a progressive reduction in the viability of P. splendens (determined by the root baiting technique) with storage of air-dried soil. Viability was completely lost within 16 days of storing air-dried soil. Although there was a slight decrease in the moisture content when moist soil was stored in polythene bags at 25-27°C, there was practically no loss of viability in P. splendens throughout the 23-day period of storage.

3.5 Relative abundance of propagules of P. splendens in 3 soils with different cropping histories

The aim of this experiment was to compare the abundance of propagules of P. splendens in three soils which had been planted to different crops. The 3 sites were located at the NIFOR Main Station.

The first site was a wet season nursery in which green and farmyard manure had been ploughed into the soil 6 months before sampling and oil palm seedlings were planted one month after the manurial treatment. Twenty cores of soil were taken from a zone of 9-12 inches below ground level. The cores of soil were bulked to form a composite sample. The second site was a portion of the nursery which had previously been planted to oil palm seedlings but had been left under a Pueraria fallow for 5 years. The composite sample from this site was also made up of twenty cores of soil. The third site was an

uncultivated soil previously under a secondary forest but cleared and planted to oil palm seedlings 3 months prior to sampling. The composite sample from this site was prepared in a similar way to the first two sites.

The soil samples were assayed for P. splendens by the root baiting technique described earlier (using 50 discs of oil palm roots for each sample). Pythium isolation percentage was calculated on the basis of the number of root discs colonised by Pythium. The results are presented in Table 14.

Table 14

Pythium isolation percentages from 3 soils with different cropping histories at the NIFOR Main Station

Soil cropping history	Pythium isolation percentage
NIFOR wet season nursery soil	50
Fallow soil under Pueraria cover crop	74
Secondary forest soil	6

The recovery value of P. splendens as determined by the root baiting technique was highest in soil under Pueraria cover and lowest in the previously uncultivated forest soil.

3.6 Effect of organic manuring on the recovery value of P. splendens from soil

The incorporation of organic manure with nursery soil is a standard

cultural practice in oil palm cultivation at the NIFOR Main Station. The effect of green and farmyard manuring on the recovery value of Pythium from soil was investigated.

A portion of the nursery land which had been left fallow under leguminous cover for 5 years was sampled at weekly intervals before, during and after the green leguminous cover and farmyard manure were ploughed into the soil. Farmyard manure was ploughed into the soil at the rate of 50 tons per acre. Soil samples were taken from a zone of 9–12 inches below ground level and ten such samples were bulked to form a composite sample. The first sample (Sample A) was taken when the soil was under leguminous cover. The second sample (Sample B) was taken after the leguminous cover had been ploughed into the soil. The third sample (Sample C) was taken after farmyard manure in addition to the green manure had been ploughed into the soil. The fourth sample (Sample D) was taken after oil palm seedlings had been planted in the amended soil. For the control, soil samples were taken from a portion of the nursery which was under leguminous cover throughout the duration of the experiment. The isolation of Pythium from the soil samples was by the root baiting technique using 50 root discs for each soil sample. The recovery value of Pythium in soil was determined from the number of root discs colonised by the fungus. The results are shown in Table 15.

Table 15

The recovery values of P. splendens from soil amended with green and farmyard manure

Soil manurial status	Pythium isolation percentage	
	Soil amended with organic manure	Non-amended soil (control)
A	74	74
B	64	74
C	96	72
D	94	74

A = Soil under leguminous cover crop

B = Soil into which only leguminous cover crop had been ploughed

C = Soil into which green and farmyard manure had been ploughed

D = Soil amended with organic manure and planted to oil palm seedlings

There was a slight decrease in the recovery value of P. splendens (as determined by the root baiting technique) soon after the incorporation of green manure with the soil. As the green manure decomposed and following the incorporation of farmyard manure with the soil, there was a sharp increase in the recovery index of P. splendens. There was no marked variation in the recovery values of the fungus from the non-amended soil under the leguminous cover crop.

3·7 Comparative recovery values of P. splendens from soil around the roots of healthy and diseased oil palm seedlings

To find out whether there were local differences in the recovery values of P. splendens from nursery soil at the NIFOR Main Station, the recovery value of the fungus from soil (as determined by the root baiting technique) around the roots of oil palm seedlings affected by the blast disease was compared with that from soil around the roots of healthy oil palm seedlings.

Columns of soil 12 inches deep were taken with a tubular sampler from five points from which healthy oil palm seedlings were pulled up. The samples were bulked to form a composite sample. A similar composite sample was prepared from five points where oil palm seedlings affected by blast were pulled up. The soil samples were assayed for P. splendens by the root baiting technique. The percentage of root discs colonised by the fungus was taken as a measure of the recovery value of the fungus from the soil sample. The results are given in Table 16.

Table 16

The recovery values of P. splendens from soil around the roots of healthy and diseased oil palm seedlings

Replicates	Isolation of P. splendens			
	Soil around the roots of healthy seedlings		Soil around the roots of diseased seedlings	
	No. of baits with fungus	Percentage	No. of baits with fungus	Percentage
1	3	60	4	80
2	0	0	4	80
3	1	20	4	80
4	0	0	4	80
Average	1	20	4	80

The recovery value of P. splendens from the soil around the roots of seedlings affected by the blast disease was 4 times higher than that from the soil around the roots of healthy seedlings.

3·8 Summary of results on the ecological studies on P. splendens

The most suitable method of isolating P. splendens from soil was by burying discs of live oil palm roots in soil for 24 hours and then plating the colonised root discs on cassava-dextrose agar.

By using the root baiting technique, the infective propagules of P. splendens were found to be more abundant at a soil depth of 9 inches than at other soil depths between 1-24 inches below ground level.

The highest recovery value of P. splendens, as determined by the root baiting technique, was found to be in August-October and the lowest value in November-January. These variations in the recovery of P. splendens corresponded with variation in soil moisture.

The viability of P. splendens (as determined by the root baiting technique) decreased with loss of soil moisture and also with storage of air-dried soils. The recovery value of the fungus was high in cultivated and low in non-cultivated soils. There was also an increase in the recovery value of the fungus following the incorporation of organic manure with soil. The recovery value of the fungus was 4 times higher in soil around the roots of diseased oil palm seedlings than around the roots of healthy seedlings.

4:0 CULTURAL STUDIES ON P. SPLENDENS AND R. LAMELLIFERA

This section deals with the effects of different types of agar medium, temperature, compound fertilizer and fungicides on the linear growth, sporulation and germination in P. splendens and R. lamellifera which together cause the blast disease.

4·1 Effect of the types of agar medium on the linear growth, sporangial production and germination in P. splendens

For investigating the effect of the types of agar medium on the rate of linear growth, sporangial production and germination in P. splendens, 10 ml each of Czapek-dox agar (CZDA), cassava-dextrose agar (CDA), quaker-oats agar (QOA), potato-dextrose agar (PDA), distilled-water agar (DWA), soluble-starch agar (SSA), V8-juice agar (V8JA), corn-meal agar (CMA) and malt-extract agar (MEA) were poured into separate sterile petri dishes and allowed to set. The plates were inoculated at the centre with 0·4 cm-diameter mycelial discs taken from the edge of a 26 hour-old culture of P. splendens. There were three replicates for each medium and all the inoculated plates were incubated at 26°C. Linear growth and sporangial production were determined as described under "Materials and Methods".

For investigating sporangial germination in P. splendens, 2 ml of a sporangial suspension prepared from 4-day-old inoculated pawpaw fruits were pipetted and spread over the surface of the solidified agar medium in each

plate . There were two replicates for each medium and all the inoculated plates were incubated at 26°C . Germination was determined as described earlier (Section IV) . The results obtained are shown in Table 17 and illustrated in Fig. 5 .

Table 17

Linear growth, sporangial production and germination in P. splendens on different types of agar medium

Agar media	Mean increase in colony diameter after 24 hours of incubation (cm)	Mean no. of sporangia/microscopic field after 7 days of incubation	Sporangial germination after 3 hours of incubation (%)
CDA	7·4	31·0	57
CMA	5·2	13·8	63
CZDA	7·6	86·4	53
DWA	6·1	3·1	58
MEA	1·7	13·5	5
PDA	6·8	28·8	49
QOA	6·9	4·2	74
SSA	6·1	5·6	55
V8JA	5·8	18·8	49

(a) Linear growth

Czapek-dox and cassava-dextrose agar were found to be the best media

for the linear growth in P. splendens having over 4 times the growth rate on malt-extract agar which was the least suitable medium for linear growth.

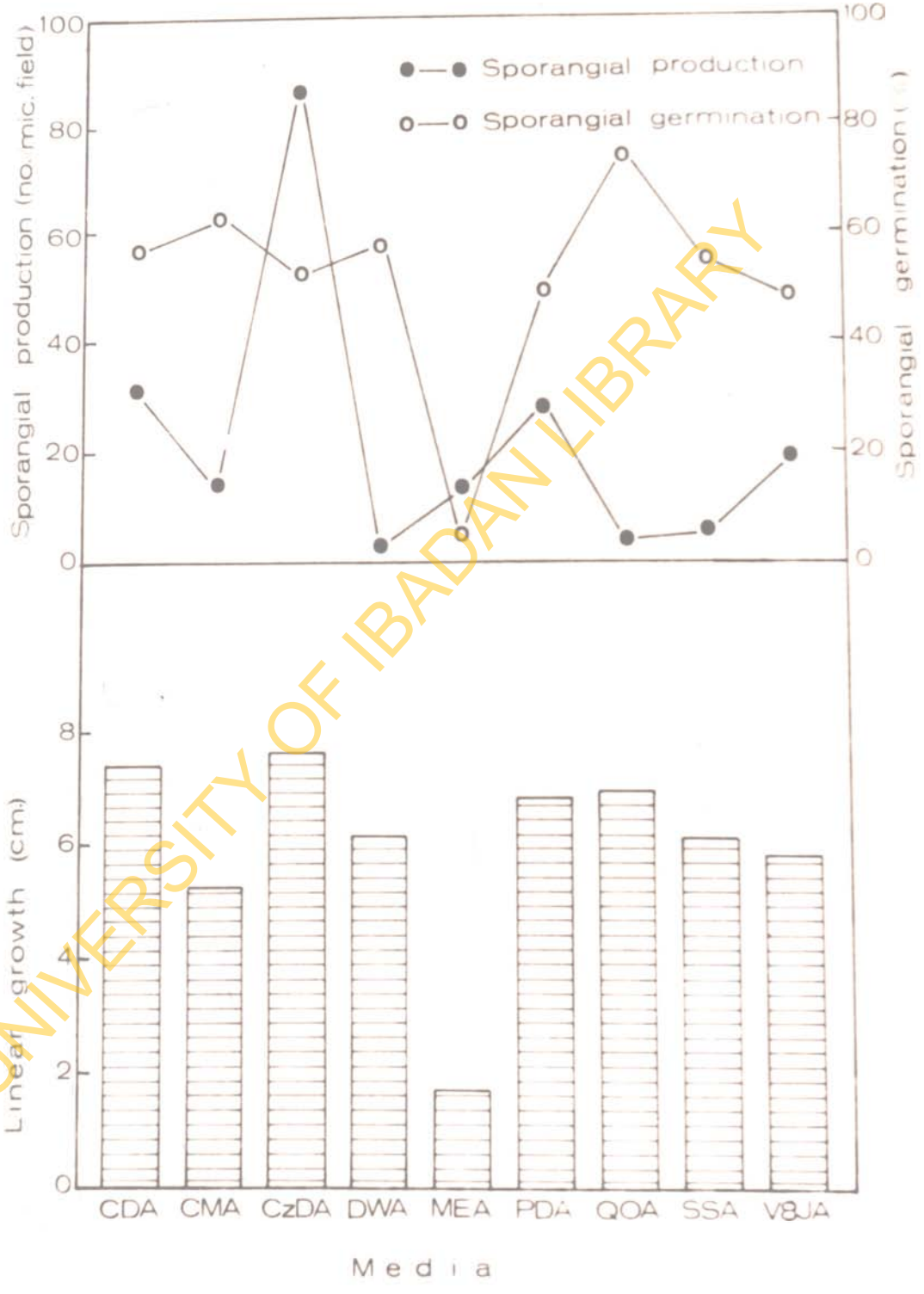
(b) Sporangial production

Czapek-dox agar which supported the greatest growth in P. splendens was also found to be the most suitable medium for sporangial production. The mean number of sporangia after 7 days of incubation being 28 and 21 times that on distilled-water agar and quaker-oats agar respectively. Distilled-water agar was the worst medium for sporangial production (Fig. 5).

(c) Sporangial germination

The greatest sporangial germination in P. splendens was observed on quaker-oats agar and the least on malt-extract agar (Fig. 5).

Fig. 5. Linear growth, sporangial production and germination in P. splendens on different types of agar medium.



4·2 Effect of temperature on the linear growth and sporangial germination in *P. splendens*

The aim of this experiment was to find out the most suitable temperature for the linear growth and sporangial germination in *P. splendens*.

Plates containing 10 ml of solidified cassava-dextrose agar were inoculated at the centre with 0·4 cm-diameter mycelial discs taken from the periphery of 4 day-old colonies of *P. splendens*. Three inoculated plates were incubated at the following constant temperatures: 15°, 20°, 25°, 30°, 35° and 40°C.

For sporangial germination, plates of cassava-dextrose agar were inoculated with sporangial suspension as described in Section IV and three of the plates were incubated at the various temperatures. Linear growth and sporangial germination were determined as described earlier (Section IV). The results obtained are presented in Table 18 and illustrated in Fig. 6.

Table 18

Linear growth and sporangial germination in P. splendens at constant temperatures ranging from 15° - 40°C

Temperature (°C)	Mean increase in colony diameter after 24 hrs of incubation (cm)	Sporangial germination after 5 hrs of incubation (%)
15	0·6	0
20	2·8	17
25	5·4	97
30	5·6	96
35	0·0	8
40	0·0	0

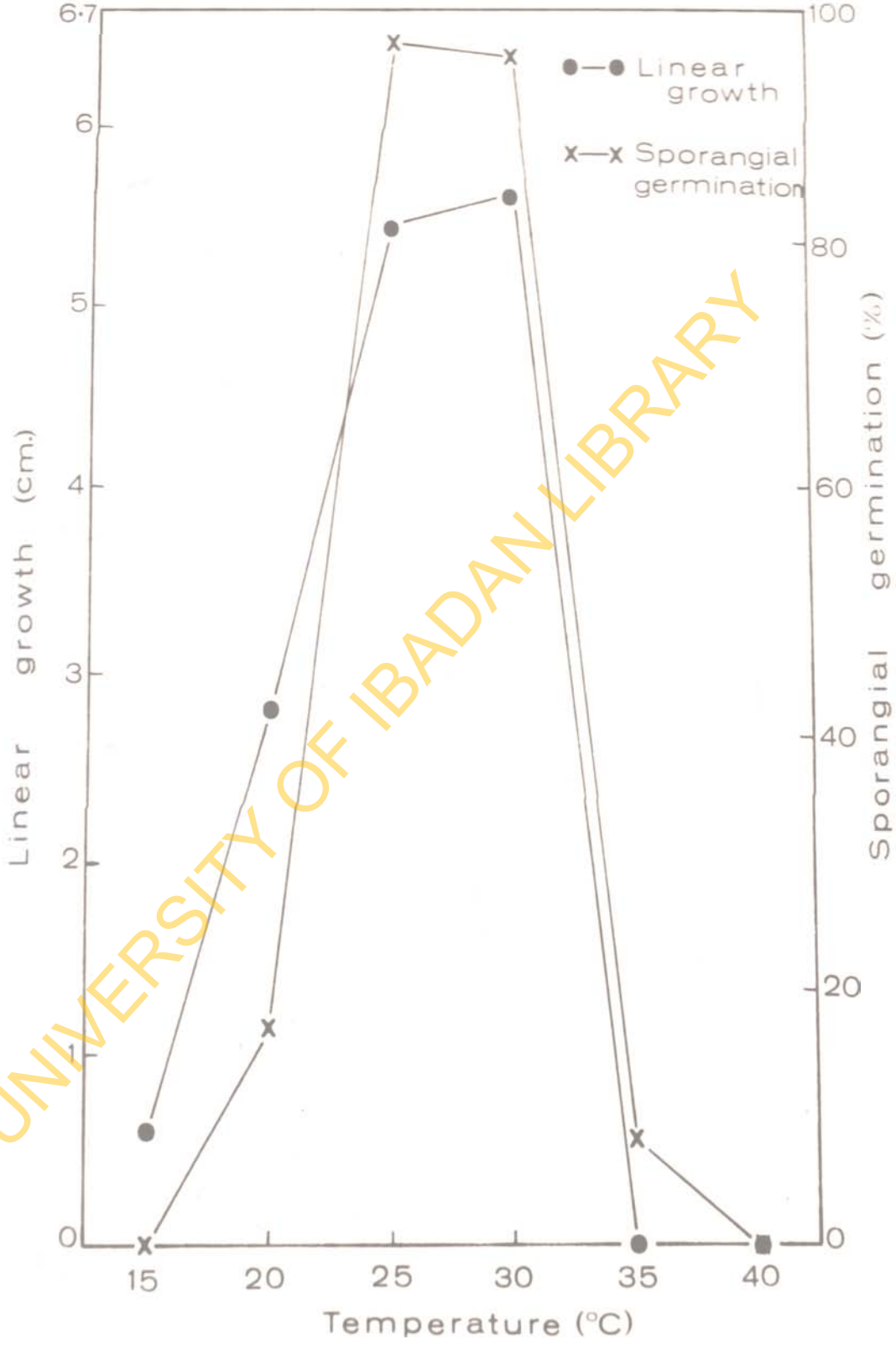
(a) Linear growth

A temperature range of 25°-30°C was found to be most suitable for the linear growth in P. splendens. Linear growth was higher at 30°C being nearly 4% higher than at 25°C. The fungus did not grow at all at 35°C or 40°C during the duration of this experiment (Fig. 6).

(b) Sporangial germination

A temperature range of 25°-30°C was also found to be the most suitable for sporangial germination in P. splendens. There was no significant difference between sporangial germination at 25°C and at 30°C. Sporangial

Fig. 6. Linear growth and sporangial germination in *P. splendens* on cassava-dextrose agar at different temperatures.



germination was low at 20°C and lower still at 35°C . The sporangia of the fungus did not germinate at the low and high temperatures of 15°C and 40°C during the 5 hour-duration of the experiment .

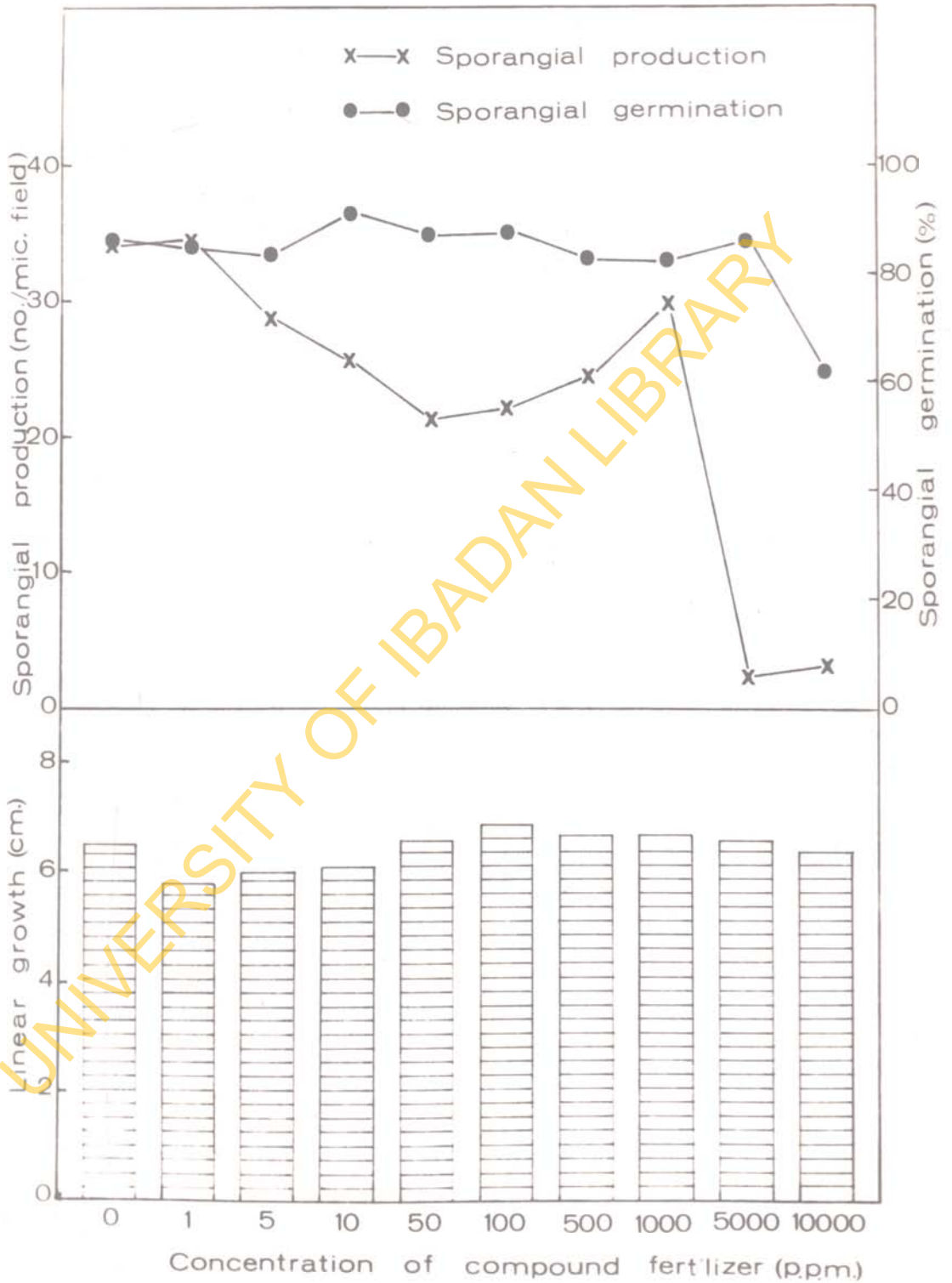
4·3 Effect of compound fertilizer on the linear growth, sporangial production and germination in P. splendens

The effect of compound fertilizer (composed of NPKMg in the ratio of 1:1:1:2) which is normally applied routinely in the cultivation of the oil palm on the growth, sporangial production and germination in P. splendens was investigated .

Cassava-dextrose agar was impregnated with the compound fertilizer as described earlier (Section IV). The final concentrations of the compound fertilizer in the agar medium were 0, 1, 5, 10, 50, 100, 500, 1,000, 5,000 and 10,000 p.p.m. The plates were then inoculated at the centre with 0·4 cm-diameter mycelial discs taken from the edges of 31 hour-old colonies of P. splendens . There were three replicates for each concentration and the inoculated plates were incubated at 26°C .

1 ml of a sporangial suspension from a 23 day-old culture of P. splendens grown on cassava-dextrose agar was also pipetted and spread over the surface of the fertilizer-amended agar medium . There were two replicates for each level of fertilizer and all the inoculated plates were incubated at 26°C . Sporangial germination was assessed after 3 hours of

Fig. 7. Linear growth, sporangial production and germination in P. splendens on cassava-dextrose agar impregnated with different concentrations of compound fertiliser (NPKMg).



incubation. The results obtained from these two experiments are shown in Table 19 and illustrated in Fig 7.

Table 19

Linear growth, sporangial production and germination of P. splendens on cassava-dextrose agar impregnated with varying concentrations of compound fertilizer

Concentrations of compound fertilizer (ppm)	Mean increase in colony diameter after 24 hours of incubation (cm)	Mean no. of sporangia/microscopic field after 7 days of incubation	Sporangial germination after 3 hrs of incubation (%)
0	6·5	34·0	86·2
1	5·8	34·9	85·1
5	6·0	28·9	83·7
10	6·1	25·8	91·1
50	6.6	21·2	87·4
100	6·9	22·4	87·9
500	6·7	24·4	83·2
1,000	6·7	29·6	83·1
5,000	6·6	2·2	86·8
10,000	6·4	3·2	61·5

(a) Linear growth

At fertilizer concentrations between 1-10 p.p.m. there was a slight decrease in the linear growth in P. splendens. At higher concentrations,

the growth of the fungus was about the same as when there was no fertilizer (Fig. 7).

(b) Sporangial production

The compound fertilizer at all the concentrations tested, with the exception of 1 p.p.m., was found to inhibit sporangial production. The greatest inhibition of sporangial production was observed at 5,000 p.p.m. There was a slight increase in sporangial production at 1 p.p.m.

(c) Sporangial germination

A concentration range of between 10 p.p.m. and 100 p.p.m. was found to stimulate sporangial germination. The fertilizer at other concentrations tested in the experiment appeared to inhibit germination. The optimum stimulation was observed at 10 p.p.m. while the strongest inhibition of germination was at 10,000 p.p.m. (Fig. 7).

4·4 Induction of zoospore formation in P. splendens

Many species of the genus Pythium have been reported by other investigators to produce zoospores in vitro. Because zoospores were not observed throughout the cultural studies on P. splendens, experiments were, therefore, carried out to induce the fungus to produce zoospores.

Cultures of the fungus in cassava-dextrose agar were flooded with sterile distilled water and cooled in a refrigerator, air-conditioned room or under a fan. This technique was adopted because it had been successfully

used for Phytophthora palmivora (Butl.) Butl. which causes the black-pod disease of cocoa (Theobromae cacao L.) (Weststeijn, 1964 and 1966).

All the experiments carried out to induce the formation of zoospores in P. splendens were unsuccessful.

4·5 Induction of sexual reproduction in P. splendens

Although sexual reproduction may have occurred in P. splendens isolated from other host crops, it was not observed in the strain of the fungus which was isolated in this investigation from oil palm seedlings affected by the blast disease. Four series of experiments were, therefore, carried out to induce sexual reproduction in the fungus (P. splendens ex dura x pisifera).

In the first experiment, plates of cassava-dextrose agar, Czapek-dox agar, quaker-oats agar, potato-dextrose agar, corn-meal agar, soluble-starch agar, V8-juice agar, distilled-water agar and malt-extract agar were inoculated with mycelial discs of P. splendens and the plates were incubated at 25-27°C. The cultures were examined under the microscope for sexual structures after 2-7 days of incubation.

Sexual structures were not observed in any of the agar culture media.

In the second experiment, cultures of P. splendens in the nine types of agar medium used in the first experiment were stored at 25-27°C for two months and were then examined under the microscope for sexual organs.

Allowing the cultures to age did not induce P. splendens to undergo sexual reproduction.

In the third experiment, each of the seven isolates of P. splendens obtained from different sources was paired with P. splendens (isolated from diseased oil palm seedlings at the NIFOR Main Station) in mixed cultures. The isolates used were P. splendens ex Aden River Estates, P. splendens ex E.W.S. NIFOR 1968, P. splendens ex grove palms, P. splendens ex tenera x dura, P. splendens ex Areca catechu, P. splendens ex E.W.S F. 26 NIFOR and P. splendens ex Corozo oleifera. The medium used was corn-meal agar and the inoculated plates were incubated at 20°C. The mixed cultures were examined after 5-30 days of incubation for sexual bodies. This method also did not induce P. splendens to produce sexual bodies.

In the fourth experiment, P splendens was grown on corn-meal agar and also on a special sucrose - beta - sitosterol agar medium at 20°C. The cultures were examined for sexual structures after varying periods of incubation.

P. splendens did not produce sexual bodies within 51 days on sucrose-beta-sitosterol agar or 44 days on corn-meal agar.

4·6 Effect of certain fungicides on the linear growth and sporangial germination in P. splendens

The economic importance of the blast disease justifies research into

effective methods of the control of the disease. Chemical control is a widely used and generally effective method of controlling diseases incited by species of Pythium and Rhizoctonia. In this experiment, various chemicals were screened against P. splendens and R. lamellifera with the aim of selecting only those which are promising under laboratory conditions for nursery experimentation on the control of the blast disease.

Plates of cassava-dextrose agar were separately impregnated with 50 p.p.m. of the following commercial fungicides: Dithane M45, Captan, Dexon, Terrazole, Terraclor super x and Fernasan at concentrations of 40, 25, 35, 50, 50 and 37.5 p.p.m. of the active ingredients respectively. The first two fungicides were chosen because they have been used in the nursery in the routine control of leaf diseases of the oil palm while the remaining fungicides were selected on the basis of the work of other investigators. The plates were inoculated at the centre with mycelial discs taken from the edges of 36 hour-old colonies of P. splendens. There were three replicates for each fungicide and the plates were incubated at 30°C for 24 hours and 6 hours for the determination of linear growth and sporangial germination respectively. The percentage inhibition of linear growth and of sporangial germination were calculated from the results thus obtained. The mean values of the percentage inhibition are presented in Table 20.

Table 20

Percentage inhibition of linear growth and sporangial germination in P. splendens on cassava-dextrose agar impregnated with different fungicides

Fungicides	Percentage inhibition of linear growth after 24 hours of incubation	Percentage inhibition of sporangial germination after 6 hours of incubation
Captan	78·5	100
Dexon	38·5	94·9
Dithane M45	26.2	21·4
Fernasan	100	100
Terraclor super x	100	99·0
Terrazole	100	98·0

(a) Linear growth

Fernasan, Terraclor super x and Terrazole were found to exert the strongest inhibition on the linear growth in P. splendens. Growth inhibition was least with Dithane M45.

(b) Sporangial germination

Fernasan, Terraclor super x and Terrazole which strongly inhibited growth were also found to have the strongest action against sporangial germination in P. splendens. Dithane M45 again had the least action against sporangial germination.

Because Fernasan appeared to be the most active out of the various fungicides tested against P. splendens, it was chosen for further laboratory experiments and nursery control trials on the blast disease.

4·7 Effect of different concentrations of Fernasan on the linear growth and sporangial germination in P. splendens

After observing that Fernasan was the most active fungicide against the linear growth and sporangial production in P. splendens, it was considered necessary to study the effect of different concentrations of the fungicide on the growth and sporangial germination of the fungus so as to determine the lethal dose for 50% inhibition of linear growth and sporangial germination.

Cassava-dextrose agar was impregnated with Fernasan at the following five different concentrations: 1, 3, 5, 10 and 30 p.p.m. Three plates of each concentration were inoculated with mycelial discs taken from the edges of 24 hour-old colonies of P. splendens. Three other plates of each fungicidal concentration were inoculated with a sporangial suspension prepared from 7 day-old cultures of P. splendens for investigating sporangial germination. All inoculated plates were incubated at 30°C. Linear growth and sporangial germination were determined after 24 hours and 6 hours of incubation respectively. From the results obtained, the mean inhibition percentages of linear growth and sporangial germination were calculated. The results are shown in Table 21 and illustrated in Fig. 8.

Fig. 8. Linear growth and sporangial germination in *P. splendens* on cassava-dextrose agar impregnated with different concentrations of Fernasan.

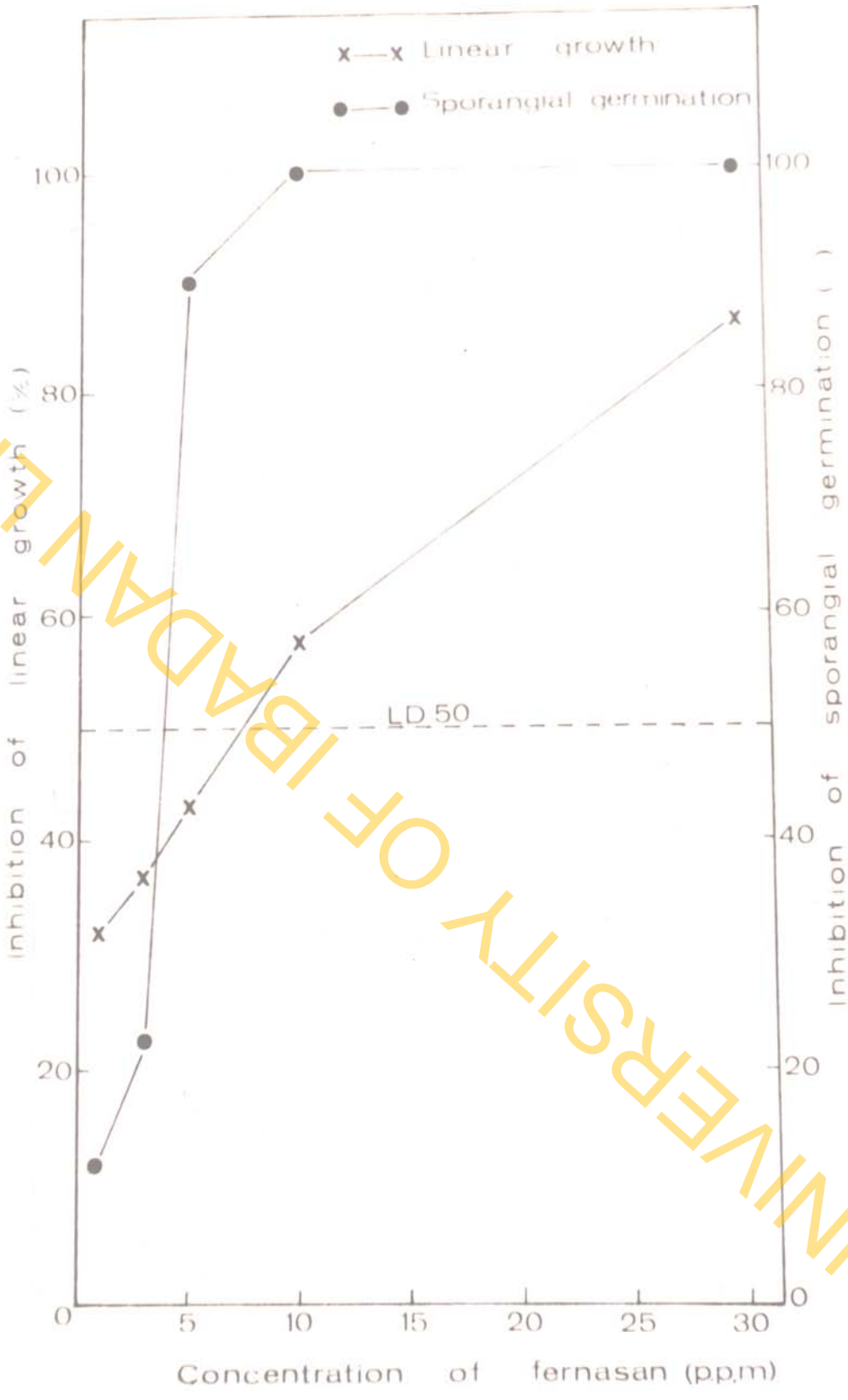


Table 21

Mean inhibition percentages of linear growth and sporangial germination in P. splendens at different concentrations of Fernasan

Concentrations of Fernasan (ppm)	Percentage inhibition of linear growth after 24 hours of incubation	Percentage inhibition of sporangial germination after 6 hours of incubation
1	32	12.2
3	37	22·4
5	43	90·0
10	57	100
30	86	100

(a) Linear growth

Inhibition of the linear growth in P. splendens increased progressively with the concentration of Fernasan (Fig. 8). Linear growth was not completely inhibited by Fernasan at the highest concentration used in the experiment (30 p.p.m). The lethal dose for 50% inhibition of the linear growth, extrapolated from Fig. 8, was found to be 7·5 p.p.m.

(b) Sporangial germination

Fernasan at the concentration of 10 p.p.m. was found to prevent sporangial germination completely in P. splendens but the same concentration of the fungicide inhibited the linear growth of the fungus by only 57%. Thus

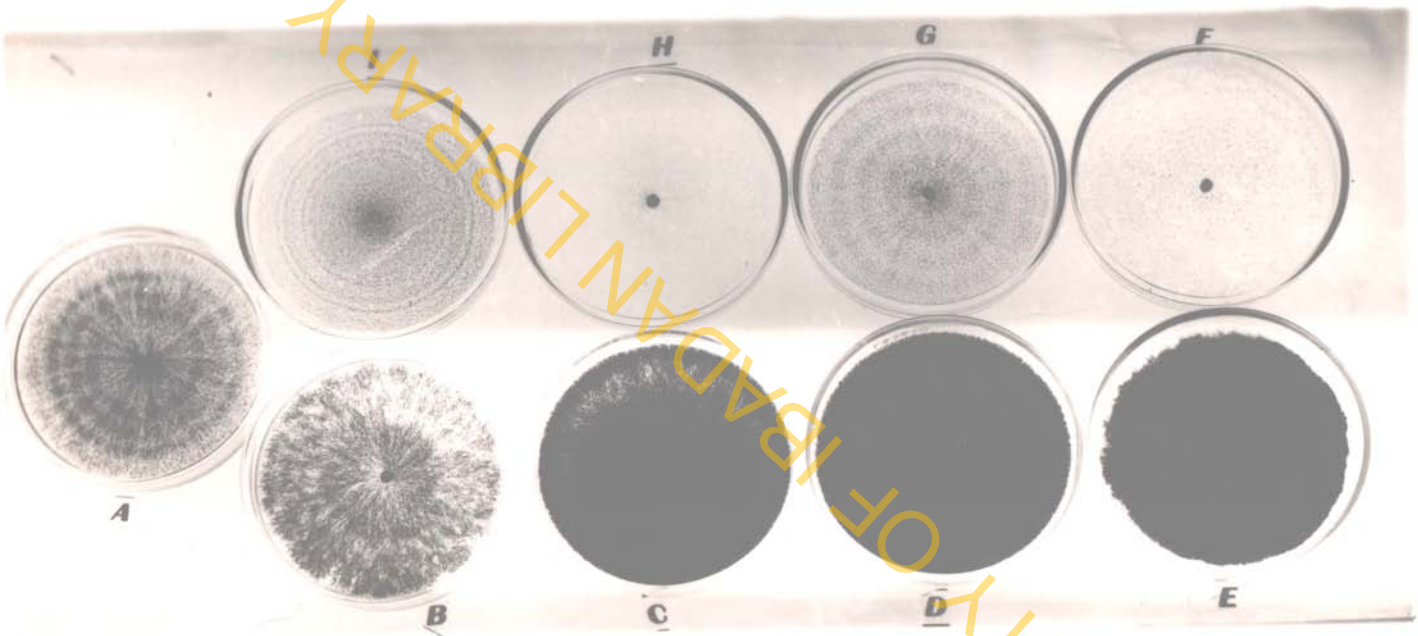
it appeared that total inhibition of the linear growth in P. splendens might require a higher concentration of Fernasan than that for total inhibition of sporangial germination. The lethal dose for 50% inhibition of sporangial germination, extrapolated from Fig. 8, was found to be 3.8 p.p.m.

4·8 Effect of the types of agar medium on the linear growth, sclerotial production and germination in R. lamellifera

For investigating the linear growth and sclerotial production in R. lamellifera in different types of agar medium, 10 ml each of Czapek-dox agar (CzDA), cassava-dextrose agar (CDA), quaker-oats agar (QOA), potato-dextrose agar (PDA), distilled-water agar (DWA), soluble-starch agar (SSA), V8-juice agar (V8JA), corn-meal agar (CMA) and malt-extract agar (MEA) were poured into separate sterile petri dishes and allowed to cool. The plates were inoculated at the centre with 0.4 cm-diameter mycelial discs taken from the edges of 45 hour-old colonies of R. lamellifera. There were three replicates for each medium and the inoculated plates were incubated at 25-27°C.

Sclerotial germination was studied by inoculating each plate with five sclerotia obtained from 37 day-old cultures of R. lamellifera grown on corn-meal agar. There were four replicates giving a total of twenty sclerotia per medium. The plates were incubated at 26°C. Data on the linear growth, sclerotial production and germination are presented in Table 22 and illustrated

Plate 11. Growth habit and production of sclerotia in Rhizoctonia lamellifera (2 weeks old) on different types of agar medium. A = soluble-starch agar; B = malt-extract agar; C = cassava-dextrose agar; D = potato-dextrose agar; E = Czapek-dox agar; F = quaker-oats agar; G = corn-meal agar; H = distilled-water agar; I = V8-juice agar.



in Fig. 9. Plate 11 shows the growth habit and production of sclerotia in R. lamellifera on different types of agar medium.

Table 22

Linear growth, sclerotial production and germination in R. lamellifera on different types of agar medium

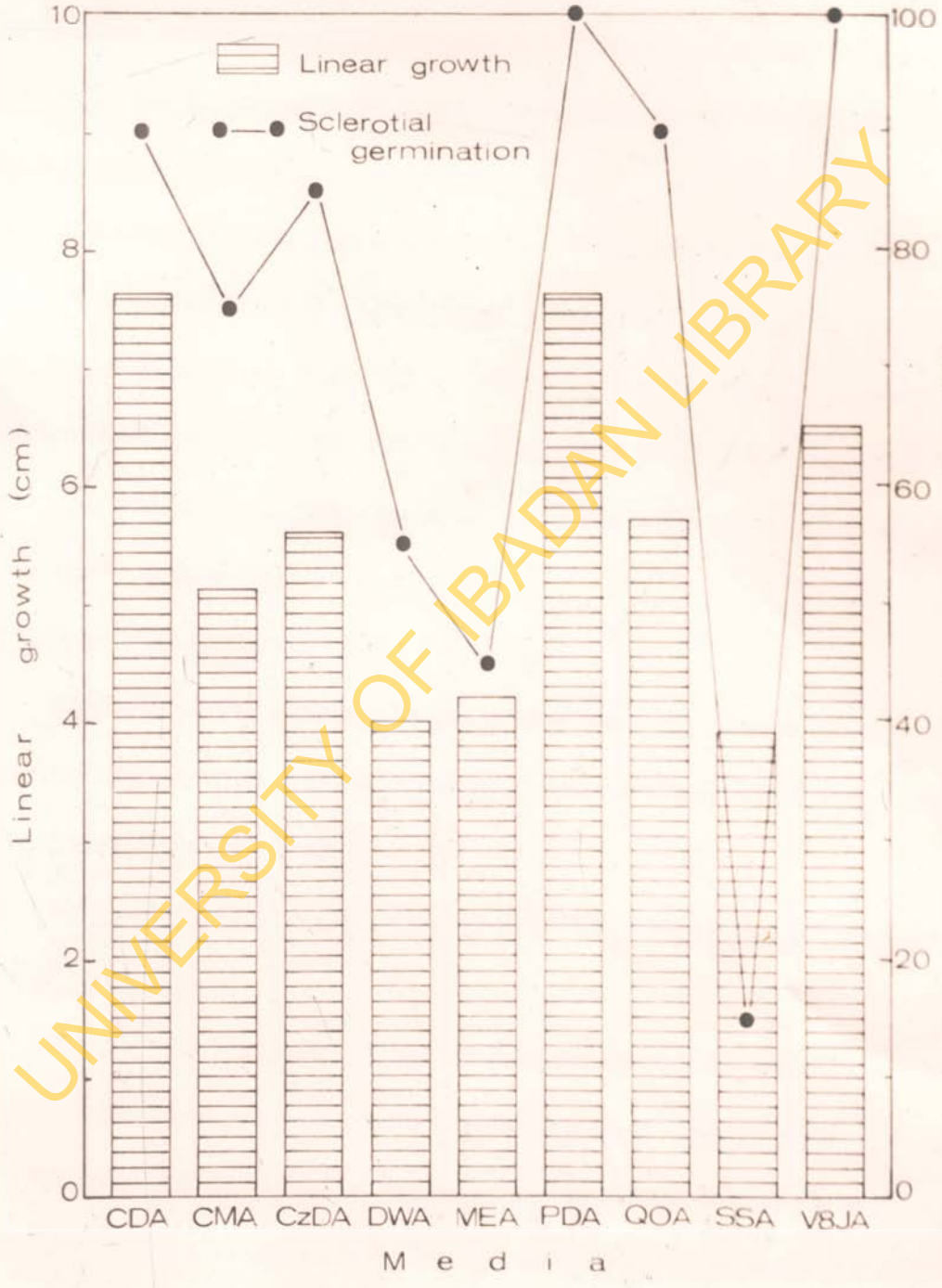
Agar media	Mean increase in colony diameter after 72 hours of incubation (cm)	Mean no. of sclerotia/ microscope field after 14 days of incubation	Sclerotial germination after 20 hours of incubation (%)
CDA	7.6	-	90
CMA	5.1	7.6	75
CzDA	5.6	-	85
DWA	4.0	1.7	55
MEA	4.2	-	45
PDA	7.6	-	100
QOA	5.7	2.3	90
SSA	3.9	4.6	15
V8JA	6.5	10.0	100

- signifies fused sclerotia

(a) Linear growth

Cassava-dextrose agar and potato-dextrose agar were found to be the best media for the linear growth in R. lamellifera. Soluble-starch agar was

Fig. 9. Linear growth, sclerotial production and germination in R. lamellifera on different type of agar medium.



the least suitable for the linear growth. Because cassava-dextrose agar appeared to be the most suitable medium for the linear growth in both P. splendens and R. lamellifera, it was used in subsequent cultural studies.

(b) Sclerotial production

V8-juice agar and corn-meal agar were found to be the best media for sclerotial production in R. lamellifera. Production of sclerotia in the fungus was lowest in distilled-water agar. Cassava-dextrose agar, Czapek-dox agar, malt-extract agar and potato-dextrose agar were found to be unsuitable for sclerotial production because the sclerotia produced in them by the fungus were fused (Plate 11).

(c) Sclerotial germination

Potato-dextrose agar and V8-juice agar were the most suitable media for sclerotial germination in R. lamellifera. Sclerotial germination was lowest in soluble-starch agar (Fig. 9).

4.9 Effect of temperature on the linear growth and sclerotial germination in R. lamellifera

The aim of this experiment was to determine the most suitable temperature for the linear growth and sclerotial germination in R. lamellifera.

Plates containing 10 ml of solidified cassava-dextrose agar were inoculated with 0.4 cm-diameter mycelial discs taken from the edges of 4 day-old colonies of R. lamellifera and incubated at 15°, 20°, 25°, 30°, 35° and

40°C. There were three replicates for each temperature. For sclerotial germination, plates of cassava-dextrose agar were inoculated with sclerotia obtained from 40 day-old cultures of R. lamellifera grown on corn-meal agar. Four plates, each of which was inoculated with five sclerotia, were incubated at each temperature. Linear growth and sclerotial germination were determined as described earlier (Section IV). The results are presented in Table 23 and illustrated in Fig. 10.

Table 23

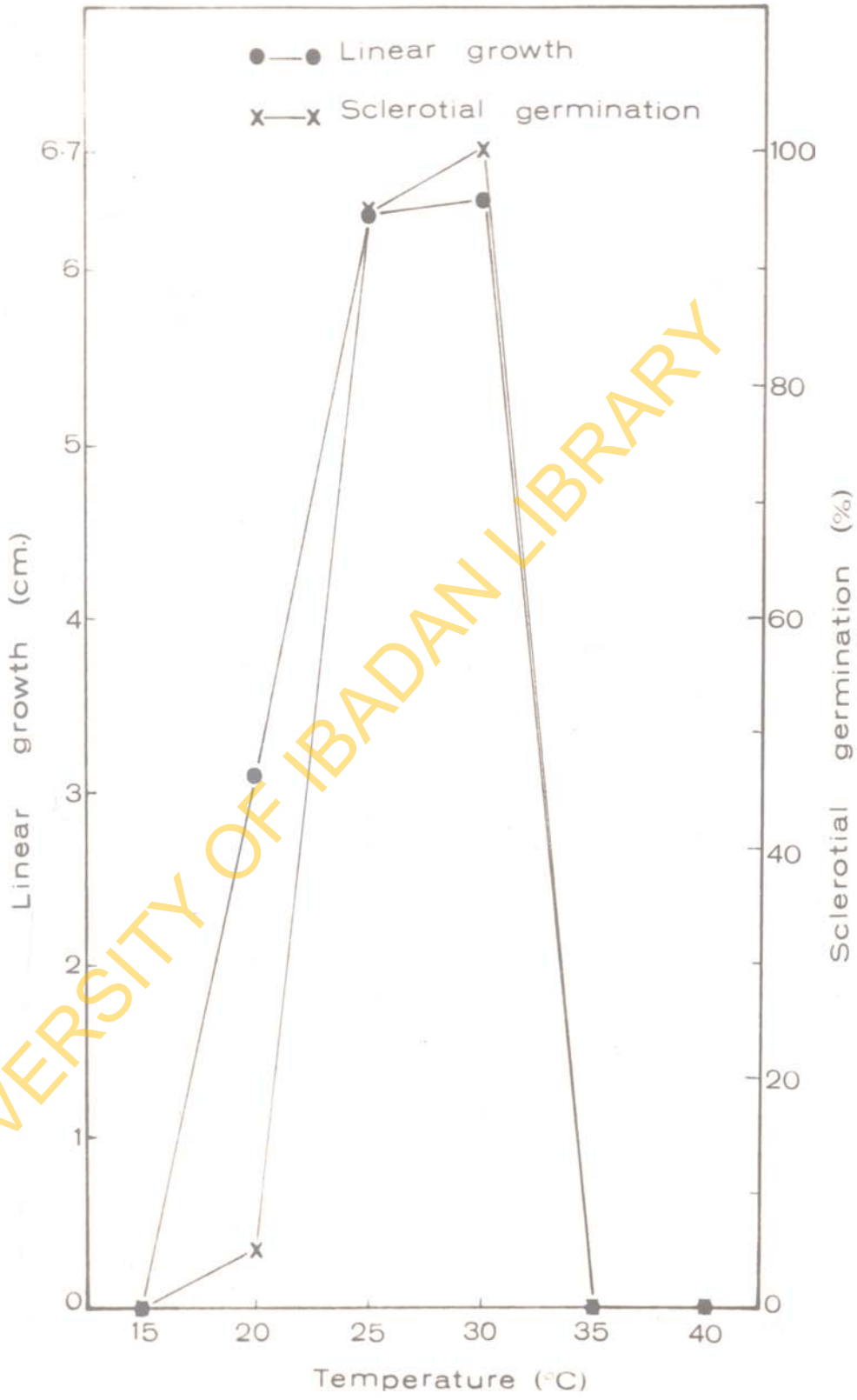
Linear growth and sclerotial germination in R. lamellifera at different temperatures

Temperature (°C)	Mean increase in colony diameter after 3 days of incubation (cm)	Sclerotial germination after 24 hours of incubation (%)
15	0.0	0
20	3.1	5
25	6.3	95
30	6.4	100
35	0.0	0
40	0.0	0

(a) Linear growth

A temperature range of 25-30°C was found to be the most suitable for the

Fig. 10. Linear growth and sclerotial germination in R. lamellifera on cassava-dextrose agar at different temperatures.



linear growth in R. lamellifera The fungus did not grow at the low and high temperatures of 15°, 35° and 40°C during the 3 day- duration of the experiment (Fig. 10).

The temperature requirements for the linear growth in R. lamellifera were about the same for P. splendens .

(b) Sclerotial germination

The same range of temperatures (25°-30°C) which was found to be most suitable for the linear growth was also the best for sclerotial germination in R. lamellifera. The sclerotia of the fungus did not germinate at 15°, 35° or 40°C during the 24 hour-duration of the experiment.

4.10 Effect of compound fertilizer on the linear growth, sclerotial production and germination in R. lamellifera

The effect of compound fertilizer (NPKMg) usually applied to promote the growth of oil palm seedlings in the nursery was investigated on the growth, sclerotial production and germination in R. lamellifera

Plates of cassava-dextrose agar and corn-meal agar were impregnated with the following concentrations of the compound fertiliser: 0, 1, 4, 10, 50, 100, 500, 1,000, 5,000 and 10,000 p.p.m. For the linear growth and sclerotial production studies, the plates were inoculated with 0.4 cm-diameter mycelial discs taken from the edges of 75 hour-old colonies of R. lamellifera grown on cassava-dextrose agar. There were three replicates for each concentration. Other

fertiliser-amended plates were inoculated with sclerotia (5 per plate) obtained from 30 day-old cultures of R. lamellifera grown on corn-meal agar for investigating sclerotial germination. There were four replicates (giving a total of twenty sclerotia) for each concentration. Inoculated plates were incubated at 25-27°C. Linear growth, sclerotial production and sclerotial germination were assessed after 63 hours, 14 days and 19 hours of incubation respectively. The results obtained are presented in Table 24 and illustrated in Fig. 11. Plate 12 shows the effect of compound fertiliser on the linear growth and sclerotial production in R. lamellifera.

Plate 12. Growth habit and production of sclerotia in Rhizoctonia lamellifera (2 weeks old) on corn-meal agar impregnated with different concentrations of compound fertiliser (NPKMg). A = 0; B = 1; C = 5; D = 10; E = 50; F = 100; G = 500; H = 1,000; I = 5,000; J = 10,000 p.p.m.

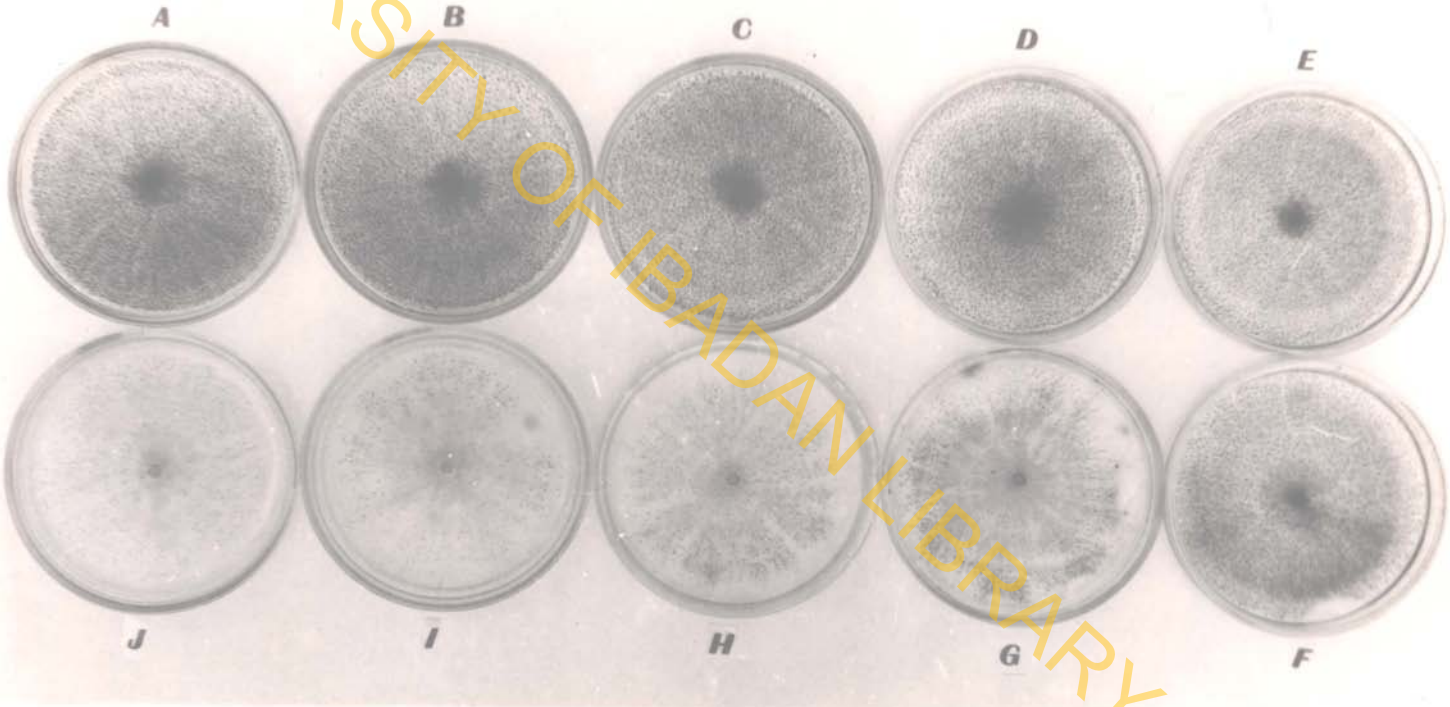


Table 24

Linear growth, sclerotial production and germination in R. lamellifera on cassava-dextrose agar amended with varying concentrations of fertilizer

Concentrations of compound fertilizer (ppm)	Mean increase in colony diameter (cm)	Mean no. of sclerotia/ microscope field on corn-meal agar	Sclerotial germination (%)
0 (control)	6.4	3.9	85
1	6.3	4.0	60
5	7.0	3.9	65
10	6.5	4.1	80
50	5.8	4.0	75
100	6.2	3.8	60
500	6.8	3.7	65
1,000	6.4	2.2	75
5,000	7.0	1.8	65
10,000	6.1	1.7	55

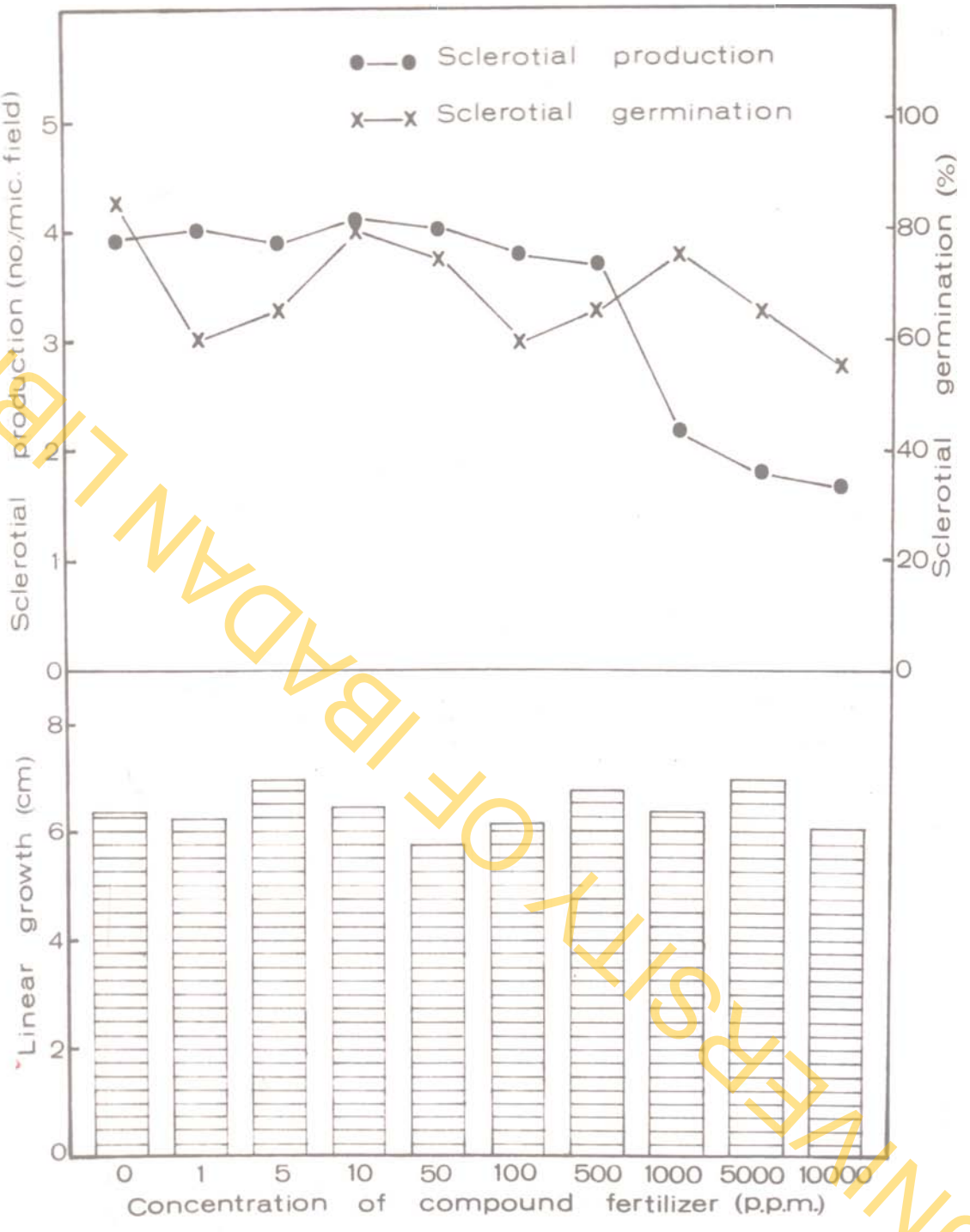
(a) Linear growth

The compound fertilizer within the concentration range of 1-10,000 ppm did not have any marked effect on the growth in R. lamellifera in comparison with the growth of the fungus in the agar medium without fertilizer (Plate 12).

(b) Sclerotial production

The fertilizer at concentrations of 1-500 p.p.m. appeared to have no

Fig. 11. Linear growth, sclerotial production and germination in R. lamellifera on agar media impregnated with different concentrations of compound fertiliser (NPKMg).



effect on sclerotial production in R. lamellifera. At higher concentrations, the compound fertilizer was found to suppress sclerotial production progressively. The strongest inhibition was observed at 10,000 p.p.m.

The effect of the compound fertiliser on sclerotial production in R. lamellifera was found to be similar to that on sporangial production in P. splendens.

(c) Sclerotial germination

The fertilizer at all the concentrations investigated was found to suppress sclerotial germination in R. lamellifera in comparison with the control. The least inhibition of sclerotial germination was observed at 10 p.p.m. and the greatest inhibition was at 10,000 p.p.m. (Fig. 11).

The compound fertiliser appeared to be more inhibitory to sclerotial germination in R. lamellifera than sporangial germination in P. splendens.

4.11 Effect of relative humidity on sclerotial longevity in R. lamellifera

The aim of this experiment was to investigate the effect of relative humidity on sclerotial longevity in R. lamellifera at a constant temperature of 20°C.

Sclerotia obtained from 37 day-old cultures of R. lamellifera grown on corn-meal agar were spread over the surface of 7 cm-diameter sterile Whatman's filter paper No. 3. One such sclerotia-laden filter paper was placed in each desiccator maintained at relative humidity values of 32, 45, 73, 84, 95 and 100%. The desiccators were placed in a room held at a

constant temperature of 20°C. The sclerotia were tested for viability at the beginning of the experiment and after varying periods of storage ranging from 19-390 days. For the viability test, twenty sclerotia were removed from a desiccator and plated (5 per plate) on potato-dextrose agar as described earlier (Section IV). The plates were incubated at 30°C. Viability was assessed in terms of the percentage of sclerotia which germinated 3-7 days after inoculation. The results obtained are shown in Table 25. Plate 13 shows the visual appearance of growth in these plates after 4 days of incubation.

Plate 13. Colonies of Rhizoctonia lamellifera (4 days old) on potato-dextrose agar. Each colony has developed from a sclerotium.

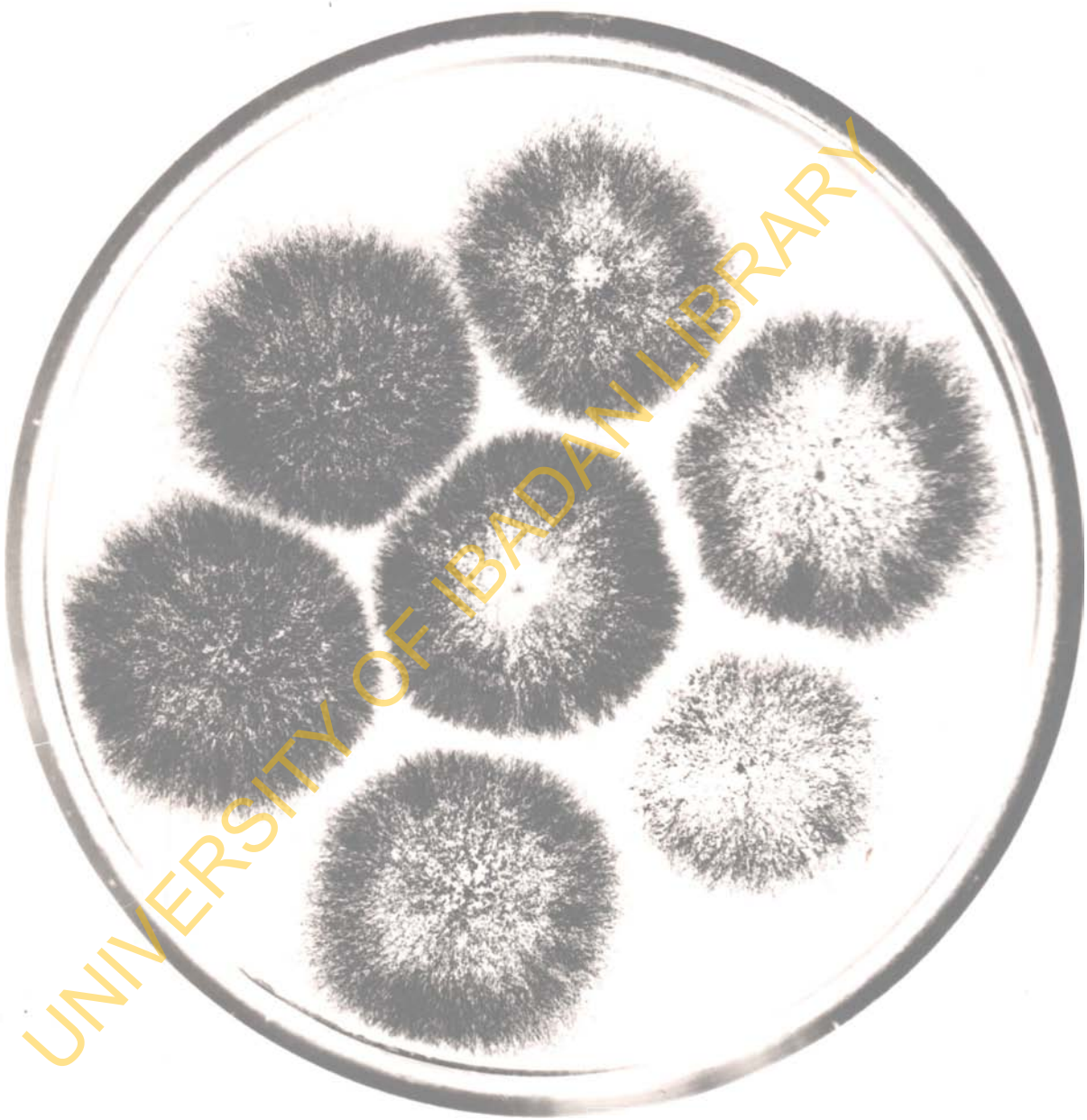


Table 25

Viability of sclerotia of R. lamellifera after varying periods of storage at different values of relative humidity

Period of storage (days)	Percentage viability/relative humidity					
	32%	45%	73%	84%	95%	100%
0	100	100	100	100	100	100
19	15	10	100	100	100	100
28	0	5	70	90	100	100
57	0	0	0	55	100	100
96	0	0	0	10	100	100
112	0	0	0	30	100	100
142	0	0	0	0	100	100
168	0	0	0	20	100	95
196	0	0	0	0	100	100
224	0	0	0	0	100	100
256	0	0	0	0	100	100
280	0	0	0	0	95	45
313	0	0	0	0	100	45
344	0	0	0	0	85	55
390	0	0	0	0	55	35

Sclerotial longevity in R. lamellifera was highest at the relative humidity value of 95%. Practically all the sclerotia of the fungus were still viable up to 256 days for 100% and up to 344 days for 95% relative humidity. At 84% relative humidity, the period for up to 90% viability was reduced to 28 days

and at 73% relative humidity only 70% viability was recorded 28 days after storage. There was a rapid loss of viability at 45% and 32% values of relative humidity and most of the sclerotia did not germinate after 19 days.

4.12 Effect of temperature on sclerotial longevity in R. lamellifera exposed to approximately 100% relative humidity.

This experiment was carried out to investigate the effect of temperature on sclerotial longevity in R. lamellifera exposed to an approximately saturated atmosphere.

Sclerotia obtained from 36 day-old cultures of R. lamellifera grown on corn-meal agar were spread over the surface of 7 cm-diameter sterile, Whatman's filter paper No. 3. One sclerotia-laden filter paper was placed in a desiccator maintained at about 100% relative humidity. A desiccator was placed in incubators held at constant temperatures of 20°, 25°, 30°, 35° and 40°C. The sclerotia were tested for viability at the beginning of the experiment and after varying periods of storage ranging from 10 to 381 days according to the methods described earlier (Section IV). The results obtained are shown in Table 26.

Table 26

Viability of sclerotia of R. lamellifera held at 100% relative humidity after varying periods of storage at different temperatures.

Period of storage (days)	Percentage viability/Temperatures				
	20°C	25°C	30°C	35°C	40°C
0	100	100	100	100	100
10	100	100	100	85	10
19	100	100	100	10	0
48	100	100	100	0	0
87	100	100	95	0	0
103	100	100	100	0	0
133	100	100	80	0	0
159	100	100	55	0	0
187	100	80	40	0	0
215	100	80	10	0	0
247	100	70	5	0	0
271	100	100	15	0	0
304	100	20	10	0	0
335	100	55	5	0	0
381	100	45	0	0	0

Sclerotia of R. lamellifera retained viability for the longest period at the temperature value of 20°C. At this temperature, there was no loss of viability for the 381 days of the experiment. The longest period for which more than 50% of the sclerotia were viable was 335 days at 25°C, 159 days at 30°C, 10

days at 35°C and less than 10 days at 40°C.

4·13 Relationship between P. splendens and R. lamellifera on agar media

The aim of this experiment was to investigate the relationship and interaction between P. splendens and R. lamellifera when the two fungi were grown in mixed cultures.

Plates of corn-meal agar, potato-dextrose agar, V8-juice agar, Czapek-dox agar, quaker-oats agar and cassava-dextrose agar were inoculated on one edge with 0·4 cm-diameter mycelial discs taken from the edges of 3 day-old colonies of R. lamellifera. Two inoculated plates of each medium were incubated at 25-27°C and two others were kept at 30°C which was found from earlier experiments to be the most suitable temperature for the growth and reproduction in P. splendens and R. lamellifera. Two days after the first inoculation, the same plates were again inoculated on the edge opposite to the R. lamellifera inocula with 0·4 cm-diameter mycelial discs taken from the periphery of 24 hour-old colonies of P. splendens. The mixed cultures of both fungi were examined under the microscope 2-14 days after the second inoculation.

Both fungi grew well on the agar culture medium. Neither the sporangium nor the hypha of P. splendens was observed to be parasitised by the hyphae of R. lamellifera.

4.14. Effect of fungicides on the linear growth in R. lamellifera

The aim of this experiment was to select only the most promising chemicals for nursery trials on the control of the blast disease.

Four fungicides (Benlate, Terraclor super x, Pentachloronitrobenzene (PCNB) and Dithane M45) and one herbicide (Gramoxone) were incorporated separately with potato-dextrose agar to a concentration of 50 p.p.m. of the commercial products. This concentration was equivalent to 25 p.p.m., 50 p.p.m., 37.5, 40 and 50 p.p.m. of the active ingredients in Benlate, Terraclor super x, PCNB, Dithane M45 and Gramoxone respectively. Gramoxone was included in the study because it is sometimes used for the control of weeds in oil palm estates. Plates of the amended potato-dextrose agar were inoculated with 0.4 cm-diameter mycelial discs taken from the edges of 4 day-old colonies of R. lamellifera grown on cassava-dextrose agar. There were three replicates for each treatment and the plates were incubated at 30°C. Linear growth was determined after 78 hours of incubation and the percentage inhibition of the linear growth was calculated from the data thus obtained. The results are shown in Table 27.

Table 27

Inhibition of the linear growth in R. lamellifera by different organic chemicals

Organic chemical	Inhibition of the linear growth (%)
Benlate	100
Dithane M45	12
Gramoxone	1
PCNB	59
Terraclor super x	61

Benlate was found to be the most active fungicide, causing the complete inhibition of the linear growth in R. lamellifera. This was followed by Terraclor super x and PCNB. Dithane M45 was very unsatisfactory. Gramoxone which is a herbicide appeared to have no effect on the linear growth of R. lamellifera.

Similar results were obtained when the experiment was repeated with corn-meal agar as the culture medium.

4.15 Effect of different concentrations of Benlate on the linear growth in R. lamellifera

Benlate was selected for further trial because it was found in Section 4.14 to have the strongest inhibitory action on the linear growth in R.

lamellifera. Preliminary experiments having shown that the linear growth in R. lamellifera was completely suppressed by Benlate at concentrations higher than 0.5 p.p.m., a range of concentrations lower than 0.5 p.p.m. was studied for the effects on the linear growth in the fungus.

Plates of corn-meal agar impregnated with Benlate to concentrations of 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.3 and 0.4 p.p.m. were inoculated with 0.4 cm-diameter mycelial discs taken from the edges of 4 day-old colonies of R. lamellifera grown on corn-meal agar. There were three replicates for each concentration and the plates were incubated at 25-27°C. Linear growth was determined after 5 days of incubation. From the figures, the percentage inhibition of the linear growth was calculated. The results are given in Table 28 and illustrated in Fig. 12.

Fig. 12. Linear growth in R. lamellifera on corn-meal agar impregnated with different concentrations of Benlate.

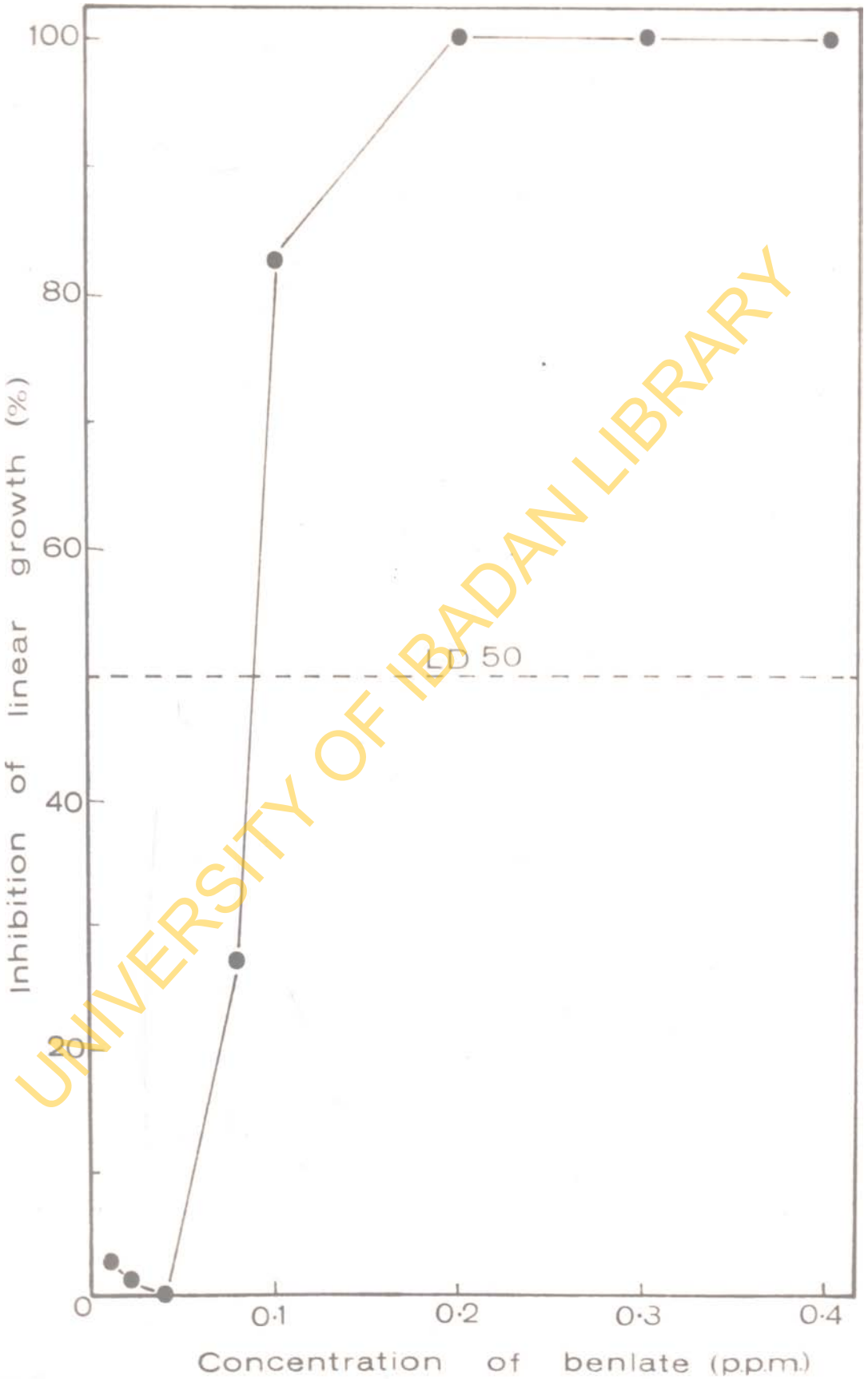


Table 28

Inhibition of the linear growth in R. lamellifera by different concentrations of Benlate

Concentration of Benlate (ppm)	Inhibition of linear growth (%)
0.01	2.7
0.02	1.7
0.04	0.0
0.08	27.0
0.1	82.4
0.2	100
0.3	100
0.4	100

Benlate at a concentration range of 0.01-0.04 appeared to have no appreciable effect on the linear growth in R. lamellifera. Growth was, however, completely inhibited by the fungicide at 0.2 p.p.m. and above. The lethal dose for 50% inhibition of the linear growth extrapolated from Fig. 12 was found to be approximately 0.1 ppm.

4.16 Summary of results on the cultural studies on P. splendens and R. lamellifera.

In vitro studies on P. splendens showed that the most satisfactory linear growth, sporangial production and germination were obtained on Czapek-dox

agar, cassava-dextrose agar at the temperature range of 25-30°C. Compound fertilizer (NPKMg) at the concentrations tested (1-10,000 ppm) appeared to have no marked effect on the linear growth but it suppressed sporangial production and germination in the fungus. Experiments carried out to induce zoospore formation and sexual reproduction in the fungus were unsuccessful. Fernasan was found to be the most active fungicide against P. splendens.

In similar cultural studies on R. lamellifera, linear growth, sclerotial production and germination were found to be most satisfactory on V8-juice agar at the temperature range of 25-30°C. Compound fertilizer (NPKMg) appeared to be inhibitory to the linear growth, sclerotial production and germination in the fungus. Sclerotial longevity in R. lamellifera was highest at 20°C and 95% relative humidity. No parasitism was observed when P. splendens and R. lamellifera were grown in mixed culture. Benlate was found to be the most active fungicide against R. lamellifera.

5.0 CULTURAL FACTORS AFFECTING THE OIL PALM BLAST DISEASE

The effects of different seasons, date of planting, soil temperature, soil moisture, soil type, soil fallowing, planting in polythene bags and colour of polythene bags on the blast disease were investigated and the results obtained are recorded under separate sub-headings.

5.1 Effect of different seasons of the year on the oil palm blast disease

The incidence of the blast disease in relation to different seasons of the year was studied among extension work seedlings (E.W.S.) which were transplanted into the wet season nursery at the NIFOR Main Station in April of every year for 5 years. Planting was done in April because this was the beginning of the rainy season in Nigeria. All the seedlings were given the usual nursery treatments described earlier (Section IV). Observations were made every month and the incidence of blast recorded. The number of seedlings affected by blast during each month was recorded and expressed as a percentage infection based on the total number of seedlings planted. The results are presented in Table 29.

Table 29

Monthly incidence of the blast disease among extension work seedlings at the NIFOR Main Station

Month of survey	Infection due to the blast disease (%)						Remarks
	1st year	2nd year	3rd year	4th year	5th year	Mean for 5 years	
April	0.0	0.0	0.0	0.0	0.0	0.0	-
May	0.0	0.0	0.0	0.0	0.0	0.0	-
June	0.0	0.0	0.0	0.0	0.0	0.0	-
July	0.0	0.0	0.0	0.0	0.0	0.0	-
August	0.0	0.0	0.0	0.0	0.0	0.0	-
September	0.0	0.0	0.0	0.0	0.0	0.0	-
October	1.7	0.0	0.6	0.0	0.6	0.6	++
November	11.7	2.0	3.8	5.6	4.4	5.5	+++
December	1.7	6.5	6.6	2.6	4.2	4.3	+++
January	1.7	2.5	1.8	0.0	3.0	1.8	++
February	0.0	1.0	0.8	0.4	0.5	0.5	++
March	0.0	0.5	0.2	0.0	0.1	0.2	+
Overall blast	16.8	12.5	13.8	8.6	12.8	12.9	

- no incidence of the blast disease
+ negligible incidence of the blast disease
++ low incidence of the blast disease
+++ high incidence of the blast disease

The oil palm blast disease occurred between October and March of every year (during the dry season). No incidence was recorded between April and September (during the rainy season). The monthly incidence of the disease was similar from year to year in the 5 years studied.

The annual incidence of the blast disease varied between 8.6% and 16.8% with an average of 12.9%. The incidence of the disease was highest in November and December during which more than 60% of the total annual incidence was recorded.

Apart from the oil palm, observations were also made on other palms; these were deli dura palms (Deli x Deli), date palm (Phoenix dactylifera Linn.), royal palm (Roystonea regia (H.B.K.) Cook) and arecanut palm (Areca catechu Linn.). The percentage of infection due to the blast disease is presented in Table 30.

Table 30

Monthly incidence of the blast disease among seedlings of some other palms at the NIFOR Main Station.

Month of survey	Infection due to the blast disease (%)					Remarks
	E.W.S. (dura x pisifera)	Deli x Deli (dura x dura)	Phoenix dactylifera	Roystonea regia	Areca catechu	
April	0.0	0.0	0.0	0.0	0.0	-
May	0.0	0.0	0.0	0.0	0.0	-
June	0.0	0.0	0.0	0.0	0.0	-
July	0.0	0.0	0.0	0.0	0.0	-
August	0.0	0.0	0.0	0.0	0.0	-
September	0.0	0.0	0.0	0.0	0.0	-
October	0.6	2.7	0.0	0.0	0.0	+
November	3.8	19.2	0.0	24.4	58.5	+++
December	6.6	21.9	0.0	28.9	26.4	+++
January	1.8	1.4	0.0	6.7	9.4	++
February	0.8	0.0	0.0	8 9	0.0	++
March	0.2	0.0	0.0	0.0	1.9	+
Overall blast	13.8	45.2	0.0	68.9	96.2	

\- no incidence of the blast disease

\+ negligible incidence of the blast disease

++ low incidence of the blast disease

+++ high incidence of the blast disease

The trend of the blast disease among seedlings of other palms was similar to that among extension work seedlings (E.W.S.). Seedling mortality due to the disease was high in November and December, low in October and March and completely absent in the period of April-September. None of the seedlings of Phoenix dactylifera succumbed to the blast disease. A mortality of 96.2% was recorded among seedlings of Areca catechu. This palm was considered to be highly susceptible to the blast disease. Seedlings of Roystonea regia and Deli (dura) were also highly susceptible to the disease. Seedlings of Deli (dura), Roystonea regia and Areca catechu were found to be more susceptible to the blast disease than the oil palm (E.W.S.) itself.

5.2 Effect of date of planting on the incidence of the oil palm blast disease

Earlier workers (Bachy, 1958 and Robertson, 1959a) have associated the time of the year when oil palm seedlings are transplanted into the nursery with the incidence of the blast disease. Such an association must depend on environmental or other factors that are characteristic of these seasons in the year.

Extension work seedlings were planted in the NIFOR wet season nursery at monthly intervals from April to September. 200 seedlings were planted during each month. The seedlings were given the usual cultural treatments described earlier (Section IV). Surveys of the blast disease were carried out

at weekly intervals from October of the year of planting to March of the following year. Observations were begun in October because it was the month in which blast started to occur. The incidence of the blast disease in relation to the time of planting is shown in Table 31.

Table 31

Incidence of blast among seedlings planted at monthly intervals from April to September

Month of survey		Time of planting/No. of seedlings affected by blast					
		April	May	June	July	August	September
October	1st weekly survey	0	0	0	0	0	0
	2nd " "	0	0	0	0	0	0
	3rd " "	0	0	0	0	0	0
	4th " "	0	1	0	0	0	0
	Total for October alone	0	1	0	0	0	0
November	1st weekly survey	0	1	0	0	0	0
	2nd " "	0	0	0	0	0	0
	3rd " "	1	0	0	0	0	0
	4th " "	3	3	2	2	0	0
	Total for November alone	4	4	2	2	0	0
December	1st weekly survey	5	6	4	6	6	0
	2nd " "	2	2	3	3	2	0
	3rd " "	3	0	9	5	3	2
	4th " "	0	0	4	5	4	0
	5th " "	3	4	5	13	12	3
	Total for December alone	13	12	25	32	27	5
January	1st weekly survey	3	3	4	4	9	4
	2nd " "	1	2	1	7	9	2
	3rd " "	0	0	0	2	1	0
	4th " "	1	1	2	4	3	2
	Total for January alone	5	6	7	17	22	8
February	1st weekly survey	1	2	4	4	3	6
	2nd " "	0	0	0	0	1	3
	3rd " "	0	2	1	0	1	1
	4th " "	1	2	2	1	0	2
	Total for February alone	2	6	7	5	5	12
March	1st weekly survey	0	0	0	0	1	1
	2nd " "	0	0	0	0	0	0
	3rd " "	1	0	0	1	0	0
	4th " "	0	0	1	0	0	1
	Total for March alone	1	0	1	1	1	2

Observations of the onset of blast in relation to the time of planting (Table 31) showed that the first incidence of the disease was observed in the 3rd week of November among seedlings planted in April; 4th week of October in May planting; 4th week of November in June and July plantings; 1st week of December in August planting and 3rd week of December among seedlings planted in September.

Apart from the isolated cases of blast in the 4th week of October and 1st week of November among seedlings planted in May and 3rd week of November among those planted in April, it appeared that the blast disease was regularly observed from the 4th week of November to about the 4th week of February of the following year.

The incidence of the blast disease was summarised for each month of planting. The number of seedlings affected by the disease was expressed as a percentage incidence. The results are given in Table 32.

Table 32

Incidence of blast in the monthly plantings of oil palm seedlings at the NIFOR Main Station

Month of survey	Incidence of blast disease (%)/Time of planting					
	April	May	June	July	August	September
October	0.0	0.5	0.0	0.0	0.0	0.0
November	2.0	2.0	1.0	1.0	0.0	0.0
December	6.5	6.0	12.5	16.0	13.5	2.5
January	2.5	3.0	3.5	8.5	11.0	4.0
February	1.0	3.0	3.5	2.5	2.5	6.0
March	0.5	0.0	0.5	0.5	0.5	1.0
Total	12.5	14.5	21.0	28.5	27.5	13.5

The seedlings with the highest total incidence of blast were those planted at the peak of the rainy season in June, July and August in which twice as many seedlings were affected as in April, May or September planting. The highest infection due to the disease was observed in December in all plantings with the exception of seedlings planted in September in which the highest incidence was recorded in February.

The incidence of the blast disease in relation to age of the seedlings in the nursery is shown in Table 33 and illustrated in Fig. 13.

Fig. 13. Relationship between the age of oil palm seedlings and the level of incidence of the blast disease in a nursery at the NIFOR Main Station.

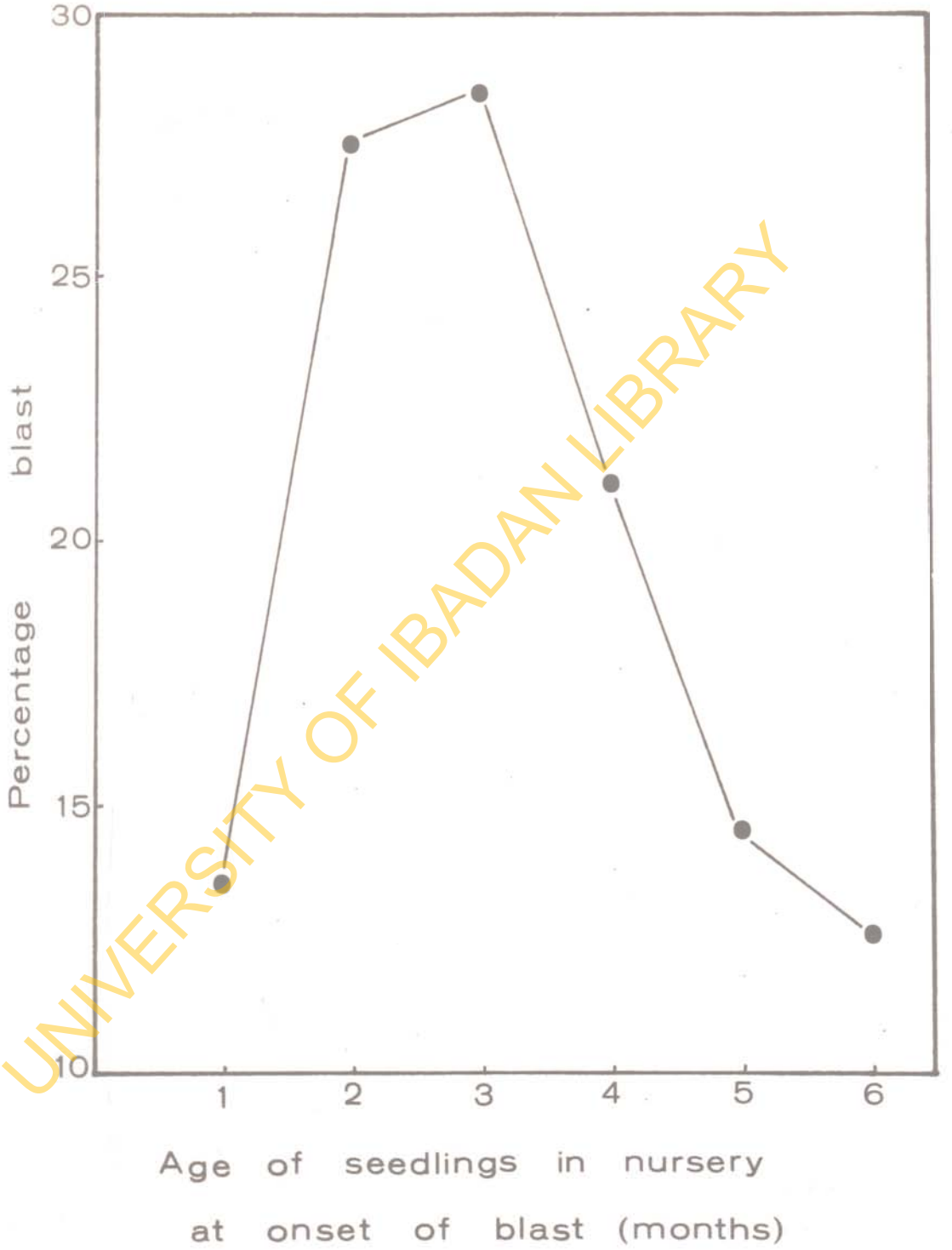


Table 33

Effect of age of seedlings in the nursery on the incidence of the blast disease

Date of planting	Age of seedlings at onset of blast in October (months)	Blast infection (%)
April	6	12.5
May	5	14.5
June	4	21.0
July	3	28.5
August	2	27.5
September	1	13.5

Seedlings transplanted into the nursery 2-4 months prior to the onset of the blast disease in October were found to be more severely affected by the disease than younger or older seedlings (Fig. 13).

5.3 Effects of soil temperature and soil moisture on the oil palm blast disease

The observation that the oil palm blast disease occurred only in the dry season as earlier recorded (Section 5.1) suggests that soil temperature and soil moisture may influence the occurrence of the disease. An experiment was, therefore, carried out to determine the effects of these factors on the incidence of the disease.

The experiment comprised eight plots each of 50 extension work seedlings.

Four plots (replications) were planted with extension work seedlings in ground beds. The remaining four plots were planted with seedlings in black polythene bags (20" x 15" layflat and 0.005" gauge) filled with non-sterilised topsoil from the NIFOR wet season nursery. Individual ground bed and polythene bag seedlings were supplied twice weekly with 4 pints and 2.5 pints of water respectively. All the seedlings were given the usual cultural treatments.

Thermometers calibrated in degrees fahrenheit were inserted vertically in soil with their bulbs 7 inches below ground level. One thermometer was installed in each plot of ground bed seedlings and one in a polythene bag per plot of polythene bag seedlings. Temperature readings were taken daily at 1400 hours and the mean values for the ground beds and polythene bags were calculated separately. The mean monthly temperature was calculated from the means of daily readings and the records were taken from May of the year of planting to March of the following year.

Soil moisture content was determined gravimetrically as described earlier (Section IV). Cores of soil were taken from 9-12 inches zone with a tubular sampler. Two such samples were taken at random from each plot and the total of eight samples were bulked to form a composite sample. Composite samples were taken thrice a month from May of the year of planting to March of the following year. The moisture content of each composite sample was determined on the day of sampling. The mean value

Fig. 14a. Relationship between soil temperature and the incidence of the oil palm blast disease in a ground bed nursery at the NIFOR Main Station.

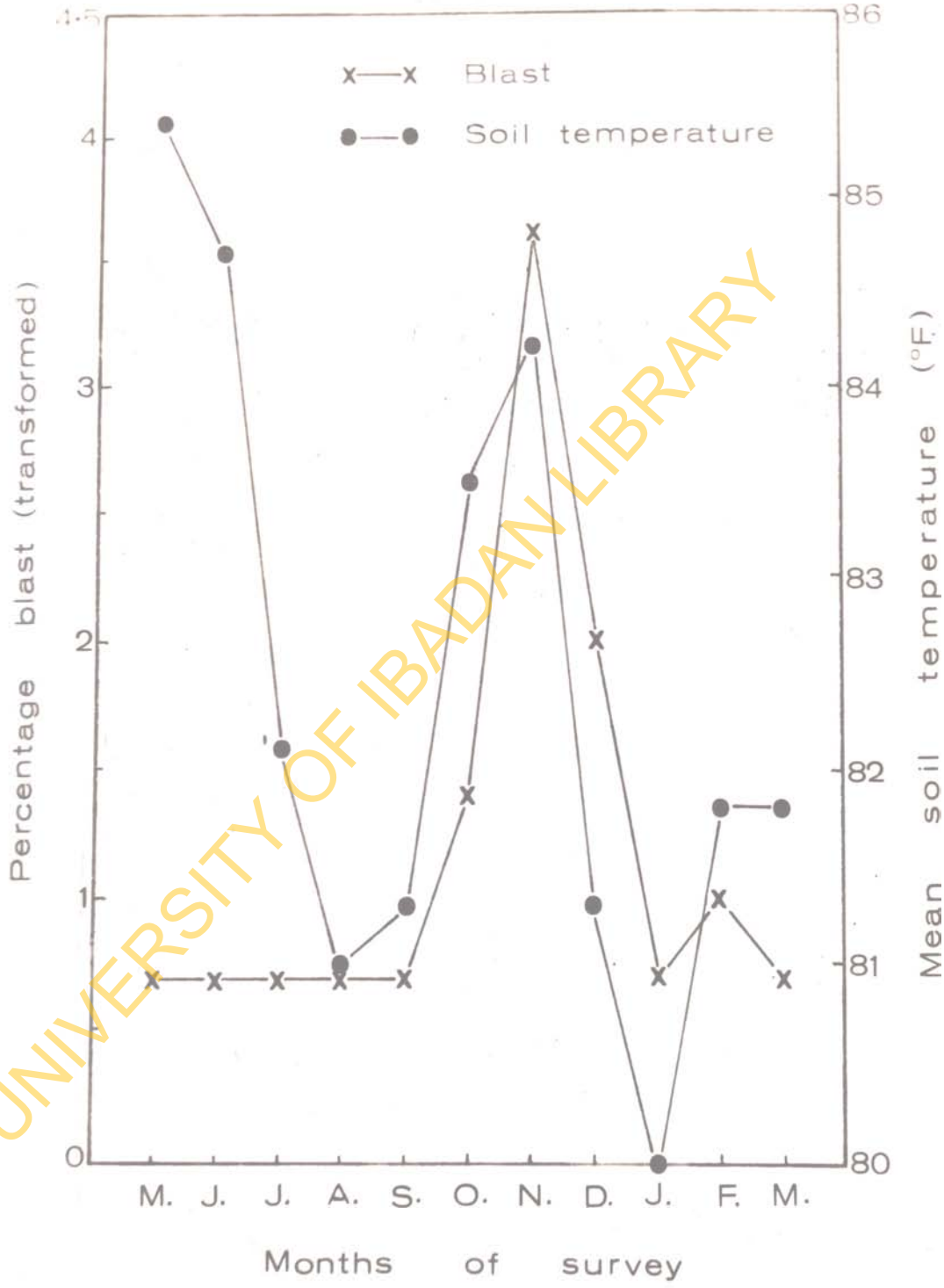
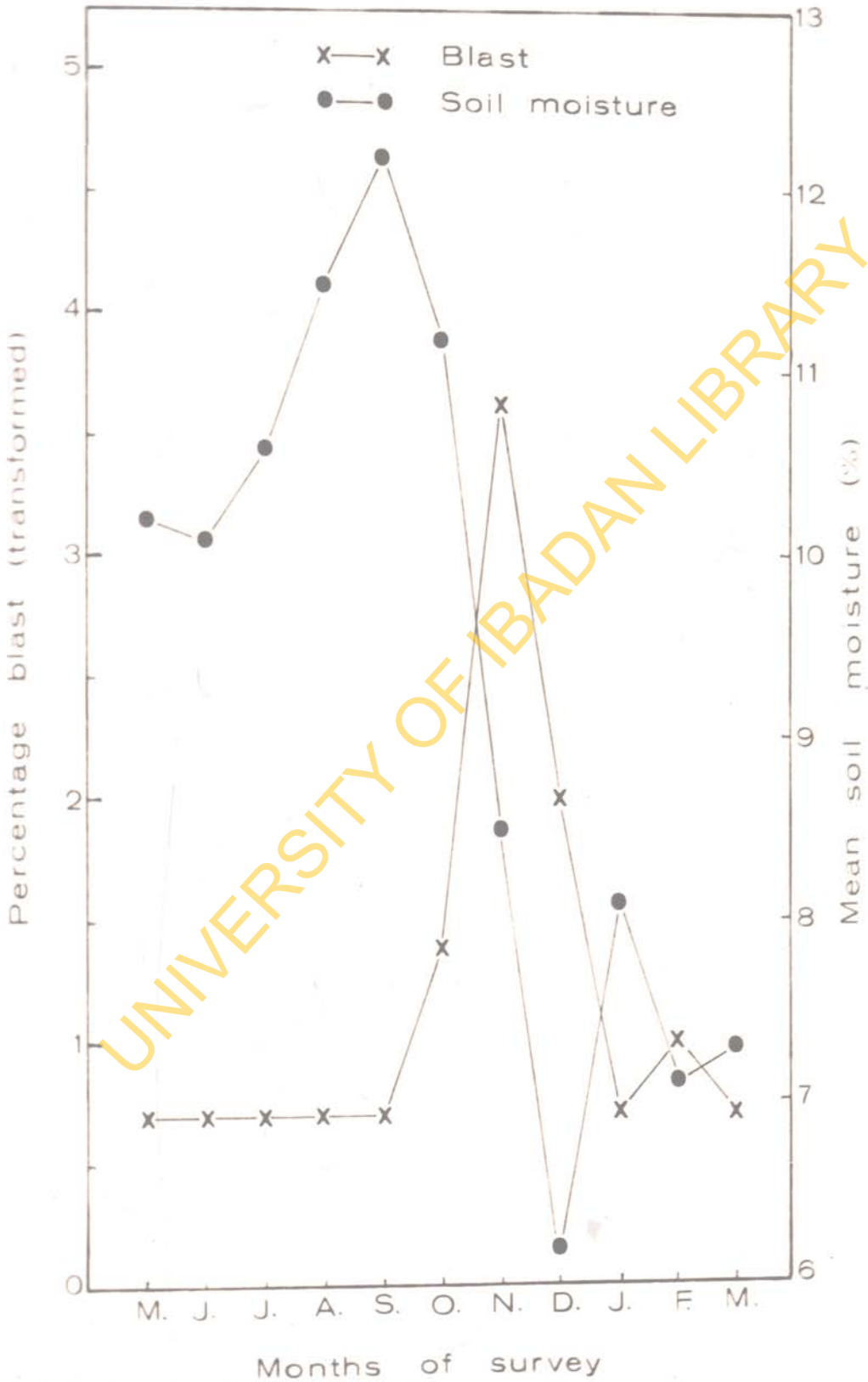


Fig. 14b. Relationship between soil moisture and the incidence of the oil palm blast disease in a ground bed nursery at the NIFOR Main Station.



for each month was calculated.

Blast disease incidence was assessed at weekly intervals from May of the year of planting to March of the following year. The number of seedlings affected by the disease in a month was expressed as a percentage infection of the total number of seedlings planted. For statistical analysis, the percentage blast was transformed as explained earlier (Section IV). Blast infection among ground bed seedlings in relation to soil temperature is illustrated in Fig. 14a.

Soil temperature appeared to be positively correlated with the blast disease (Fig. 14a). The analysis of the data, however, showed that the correlation between soil temperature and the incidence of the blast disease was not statistically significant.

Blast infection among ground bed seedlings in relation to soil moisture is also illustrated in Fig. 14b.

There was no significant correlation between soil moisture and the blast disease (Fig. 14b).

Infection due to the blast disease in relation to soil temperature in polythene bag planting is shown in Table 34 and illustrated in Fig. 15.

Fig. 15. Relationship between soil temperature and the incidence of the oil palm blast disease in a polythene bag nursery at the NIFOR Main Station.

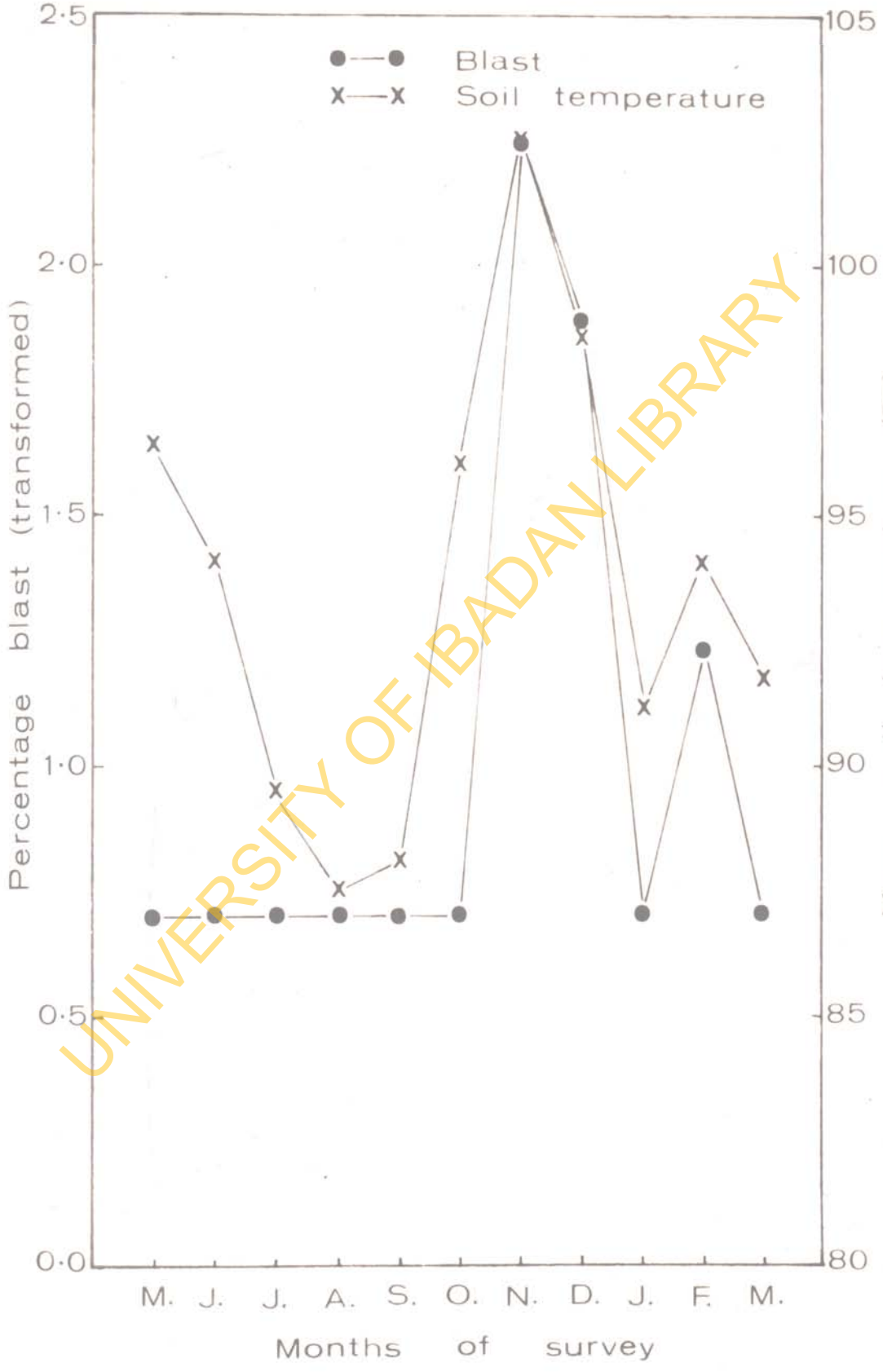


Table 34

Blast infection in polythene bag planting in relation to the monthly mean soil temperature

Months	Percentage blast (transformed)	Mean soil temperature (°F)
May	0.7071	96.4
June	0.7071	94.0
July	0.7071	89.5
August	0.7071	87.5
September	0.7071	88.0
October	0.7071	96.0
November	2.2360	102.5
December	1.8710	98.5
January	0.7071	91.1
February	1.2250	94.1
March	0.7071	91.8

Soil temperature was found to be positively correlated with the blast disease in polythene bag planting (Fig. 15). The correlation ($r = 0.7758$) was significant at the 1% level.

5.4 Incidence of the blast disease among oil palm seedlings planted in non-cultivated forest soil

All the observations which have been made so far on the blast disease have been in nurseries previously planted to the oil palm. The aim of this

experiment was to find out whether seedlings planted in freshly-cleared, secondary forest soil in which oil palm had not previously been planted would also succumb to the blast disease.

In order to investigate this, 400 extension work seedlings were planted in July (the late planting was designed to enhance the severity of the disease as observed earlier in Section 5.2) in a cleared area of Field 26 which was previously under secondary forest at the NIFOR Main Station. The same number of seedlings was planted in a cultivated wet season nursery for comparison. The seedlings planted in the non-cultivated secondary forest soil were mulched and cleared of weeds but were given no other cultural treatments. Those planted in the cultivated wet season nursery received all the usual cultural treatments. Blast incidence was assessed at weekly intervals from September of the year of planting to March of the following year. The number of seedlings affected by the disease was expressed as a percentage of the total number of seedlings planted. The results are shown in Table 35 and illustrated in Fig. 16.

Fig. 16. Incidence of the blast disease among oil palm seedlings planted in non-cultivated forest soil at the NIFOR Main Station.

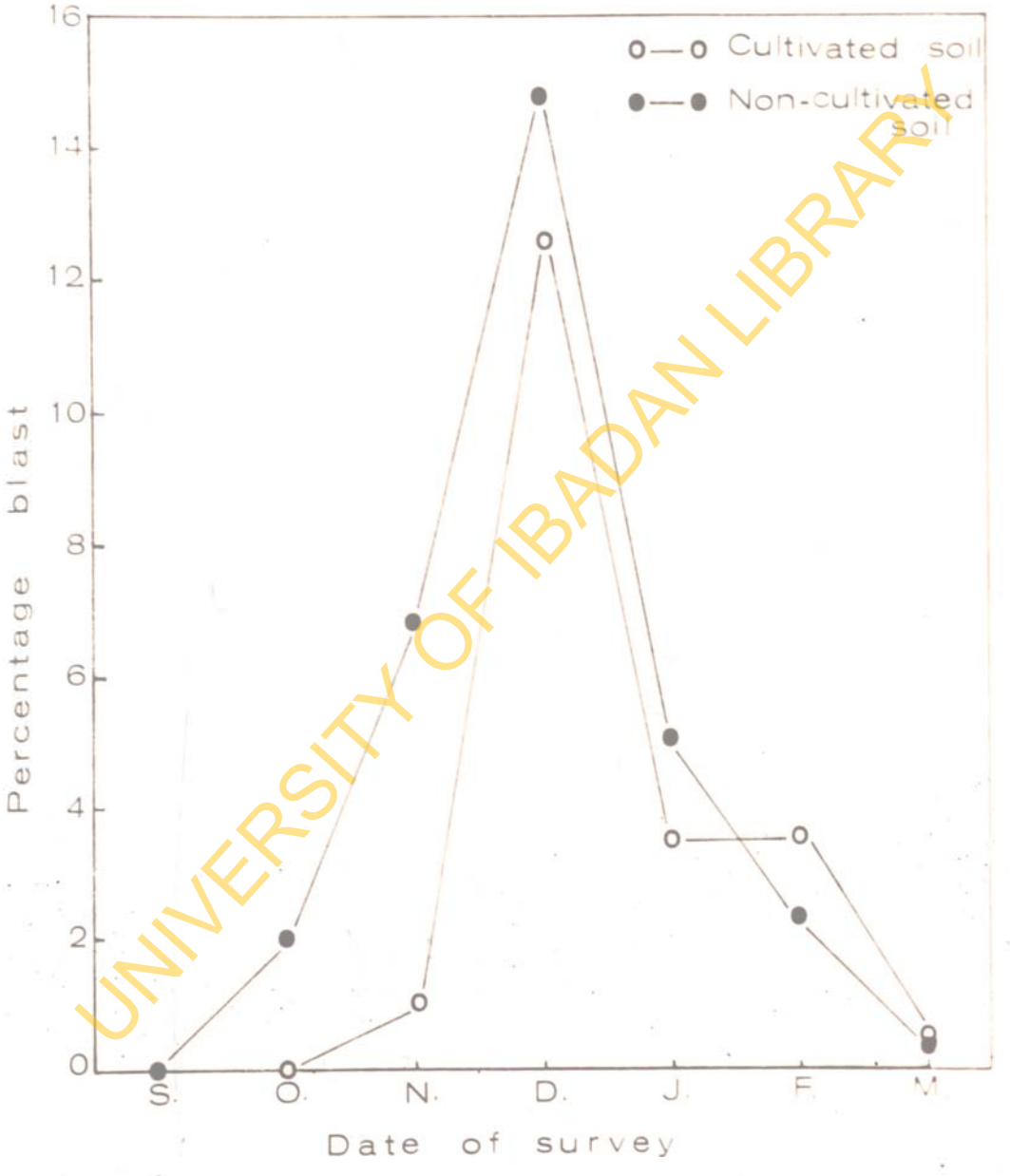


Table 35

Incidence of blast among seedlings planted in cultivated and non-cultivated soils at the NIFOR Main Station

Date of survey	Incidence of the blast disease (%)	
	Cultivated soil	Non-cultivated soil
September	0.0	0.0
October	0.0	2.0
November	1.0	6.8
December	12.5	14.8
January	3.5	5.0
February	3.5	2.3
March	0.5	0.3
Overall blast	21.0	31.2

Seedlings planted in the non-cultivated soil, like those planted in the cultivated soil, were affected by the blast disease. The incidence of the disease was higher among seedlings planted in the non-cultivated soil than those in the cultivated nursery soil (Fig. 16). The largest number of seedlings in both plantings succumbed to the disease in December.

5.5 Effect of soil fallowing on the oil palm blast disease

The nursery site at the NIFOR Main Station had been used repeatedly for more than 15 years with periods of 4-6 years fallowing under Pueraria cover

between successive crops of oil palm seedlings. The effect of using the same portion of the nursery for raising oil palm seedlings with and without intervening periods of fallow on the blast disease was investigated.

One hundred work seedlings were planted at $2\frac{1}{2}$ feet spacing in August in a portion of the nursery used in the previous year for raising oil palm seedlings (non-fallow soil). The late planting was designed to enhance the incidence of the blast disease as recorded earlier. The same number of seedlings was planted in the NIFOR wet season nursery which was last used for raising oil palm seedlings about 5 years earlier but had been left fallow thereafter under a cover of Pueraria phaseoloides Benth. (fallow soil). The cover crop together with farmyard manure was ploughed into the soil in March of the year of planting. All the seedlings were given the usual cultural treatments. Surveys of the incidence of the blast disease were carried out at weekly intervals from October of the year of planning to March of the following year. The number of seedlings affected by blast was expressed as a percentage incidence based on the total number of seedlings planted. The results are presented in Table 36.

Table 36

Incidence of the blast disease in fallow and non-fallow soils at the NIFOR Main Station

Month of survey	Incidence of the blast disease (%)	
	Fallow soil	Non-fallow soil
October	1	2
November	4	3
December	6	5
January	3	0
February	1	0
March	0	2
Overall blast	15	12

There was no marked difference in the incidence of the blast disease in both fallow and non-fallow soils. The overall incidence, however, showed that the disease was slightly more severe in the fallow than non-fallow soil.

The mean seedling height, number of leaves produced and percentage of transplantable seedlings were determined 8 months after planting the seedlings into the nursery. The results are given in Table 37.

Table 37

Mean seedling height, number of leaves produced and percentage of transplantable seedlings in fallow and non-fallow soils

Fallow status of soil	Mean height/seedling (inches)	Mean no. of leaves/seedling	Transplantable seedlings (%)
Fallow	39.7	13.2	61
Non-fallow	33.6	10.5	36

Although the overall incidence of the blast disease in the fallow soil (15%) was slightly higher than in the non-fallow soil (12%), the seedlings which were not attacked by the disease in the former were found to be taller and to produce a greater number of leaves and also a higher percentage of field transplantable seedlings than those in the latter.

5.6 Effect of topsoil and subsoil on the oil palm blast disease

Earlier experiments carried out in this investigation on the isolation of P. splendens from soil have shown that the fungus was more abundant in the 9-12 inches zone of the NIFOR nursery soil than at other zones (Section 3.1). The relationship between the distribution of the infective propagules of the fungus in soil and the incidence of the blast disease was further investigated.

Black polythene bags (17" x 16" layflat and 0.005" gauge) with three drainage holes at the base were filled with non-sterilised topsoil (the top 6

inches layer of soil) from a portion of the NIFOR nursery used in the previous year for raising oil palm seedlings. One hundred extension work seedlings from the prenursery were planted into the polythene bags in July. The same number of seedlings was planted in similar polythene bags filled with non-sterilised subsoil (soil below 6 inches depth). The polythene bags were arranged at $2\frac{1}{2}$ feet spacing in the wet season nursery at the NIFOR Main Station. All the seedlings were given the usual cultural treatments. They were surveyed for the blast disease at weekly intervals from October of the year of planting to March of the following year. The number of seedlings affected by the disease was expressed as a percentage of the total number of seedlings planted. The results are shown in Table 38.

Table 38

Incidence of the blast disease in polythene bags filled with topsoil and subsoil

Month of survey	Incidence of the blast disease (%)	
	Topsoil	Subsoil
October	0	0
November	1	2
December	4	8
January	0	3
February	0	5
March	0	0
Overall blast	5	18

Oil palm seedlings planted in the subsoil succumbed to the blast disease from November to February while those planted in the topsoil were affected by the disease only in November and December. The overall incidence of the disease in the subsoil (18%) was more than 3 times higher than that in the top-soil (5%).

For comparison of the effect of the types of soil on seedling production, the mean height, number of leaves and percentage of transplantable seedlings were determined 9 months after nursery planting. The results are given in Table 39.

Table 39

Mean seedling height, number of leaves produced and percentage of transplantable seedlings in topsoil and subsoil

Types of soil	Mean height/seedling (inches)	Mean no of leaves/ seedling	Transplantable seedlings (%)
Topsoil	36 3	9.7	90
Subsoil	34.5	9.3	77

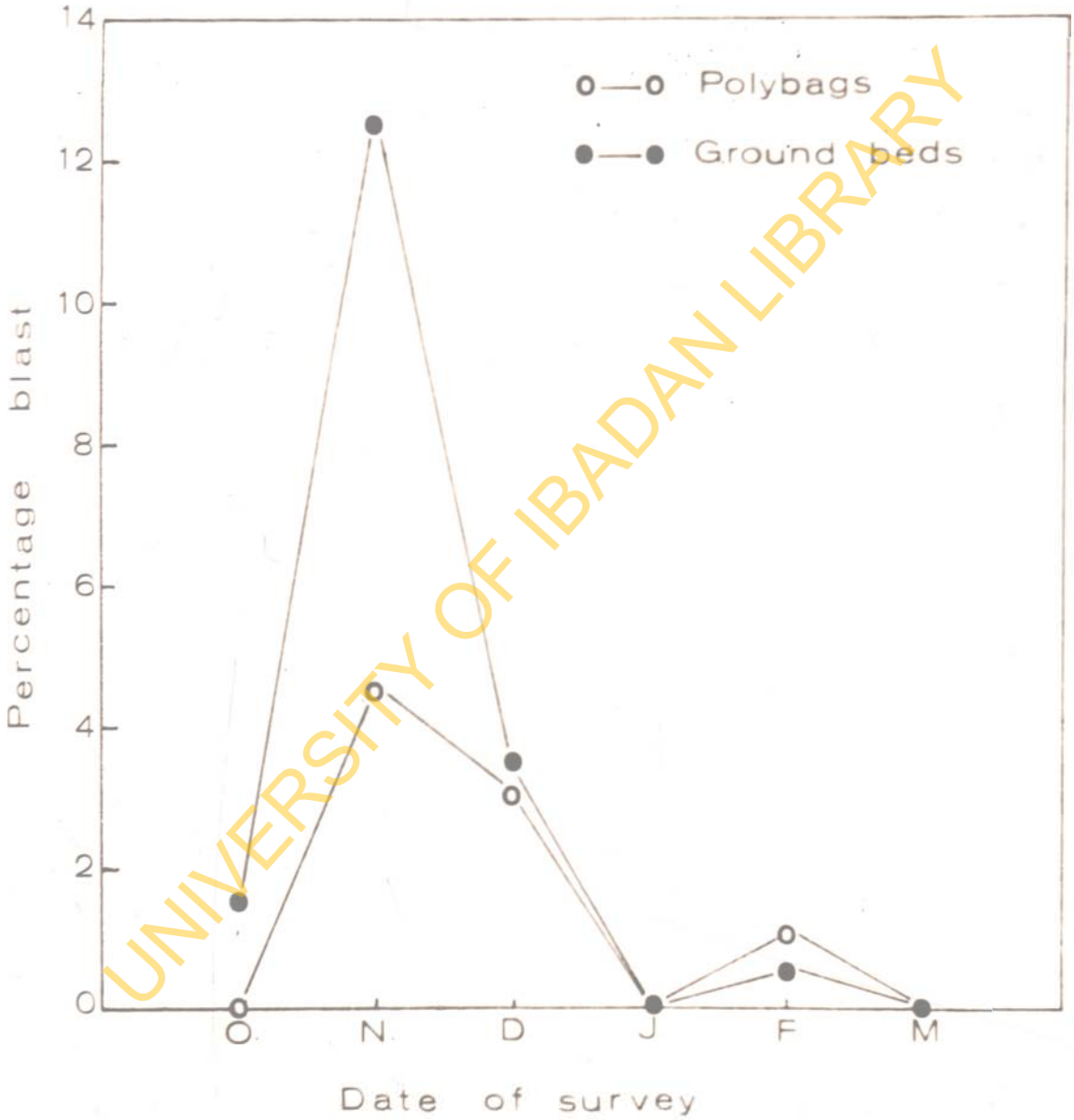
The mean seedling height, number of leaves produced and percentage of field transplantable seedlings were greater in the topsoil than in the subsoil.

5·7 Effect of planting seedlings in polythene bags on the incidence of the oil palm blast disease.

Most of the experiments carried out so far on the blast disease were in ground bed nurseries because this is the standard method of raising oil palm seedlings for field planting in Nigeria and other West African countries. In Malaysia and other countries in the Far East, however, the raising of oil palm seedlings in polythene bags for field planting is the rule rather than the exception. The incidence of blast in such countries if the disease occurs at all has been found to be much lower than in West African countries.

In order to investigate the effect of varying the method of planting by using polythene bags on the incidence of the blast disease, black polythene bags (20" x 15" layflat and 0.005" gauge) with twenty small drainage holes each 0.25 inch-diameter were filled with non-sterilised topsoil from a wet season nursery at the NIFOR Main Station. Fifty of such bags were arranged at $2\frac{1}{2}$ feet spacing in each plot and the bags were supported with 4-6 wooden pegs. There were four replicates. Extension work seedlings from the prenursery were planted singly in the polythene bags in April. The same number and type of seedlings were planted in ground beds for comparison. All the seedlings were given the usual cultural treatments. The incidence of the blast disease was determined at weekly intervals from October of the year of planting to March of the following year. The number of seedlings affected by the disease

Fig. 17. Incidence of the blast disease among oil palm seedlings planted in nursery ground beds and polythene bags at the NIFOR Main Station.



was expressed as a percentage incidence based on the total number of seedlings planted. The results are given in Table 40 and illustrated in Fig. 17.

Table 40

Incidence of the blast disease in polythene bag and ground bed plantings at the NIFOR Main Station

Month of survey	Incidence of the blast disease (%)	
	Polythene bag seedlings	Ground bed seedlings
October	0.0	1.5
November	4.5	12.5
December	3.0	3.5
January	0.0	0.0
February	1.0	0.5
March	0.0	0.0
Overall blast	8.5	18.0

The incidence of the blast disease among polythene bag seedlings was less than half of that among ground bed seedlings (Table 40). The highest incidence of the disease in both plantings occured in November during which more than 50% of the total blast incidence was recorded (Fig. 17).

5.8 Effect of the colour of polythene bags on the incidence of the oil palm blast disease.

Soil temperature was earlier found to be one of the factors which

affected the incidence of the oil palm blast disease (Section 5.3). In polythene bag nurseries, the colours of polythene bags might influence soil temperature because of differential absorption of heat. In this experiment, the effect of the colours of polythene bags on the incidence of the blast disease was investigated. Polythene bags (16" x 14" layflat and 0.005" gauge) of three colours (black, green and white)were compared for their effect on the blast disease. One hundred polythene bags of each colour were filled with non-sterilised topsoil and arranged (plots of 20 bags with 5 replications) at $2\frac{1}{2}$ feet spacing in the wet season nursery at the NIFOR Main Station. Extension work seedlings were planted singly in the polythene bags in July. The late planting was designed to enhance the incidence of the disease (Section 5.2). The seedlings were given the usual cultural treatments. Records of the blast disease incidence were taken at weekly intervals from October of the year of planting to March of the following year. The number of seedlings affected by the disease was expressed as a percentage of the total number of seedlings planted. The results are given in Table 41.

Table 41

The incidence of the blast disease among seedlings planted in polythene bags of different colours

Month of survey	Incidence of the blast disease (%)		
	Black	Green	White
October	0	0	0
November	0	0	1
December	6	10	8
January	3	0	2
February	0	2	0
March	0	1	1
Overall blast	9	13	12

The incidence of the blast disease was highest in the green and lowest in the black polythene bags but there were no marked differences that could be associated with other physical factors.

5.9 Summary of results on the cultural factors affecting the oil palm blast disease.

The oil palm blast disease was prevalent in the dry season between October of the year of planting and March of the following year. The incidence of the disease was found to be highest in November and December.

Oil palm seedlings transplanted into the nursery 2-4 months prior to the onset of the blast disease in October were found to be more severely affected by the disease than younger or older seedlings.

Soil temperature in polythene bag planting was found to be positively correlated ($r = 0.7758$) with the blast disease at the 1% level of significance. There was no statistically significant correlation between the disease and soil temperature or soil moisture in ground bed planting.

The incidence of the blast disease was lower in cultivated soil, non-fallow soil and topsoil than in non-cultivated soil, fallow soil and subsoil respectively.

The incidence of the disease was found to be lower in polythene bag than in ground bed plantings. In a comparison of the incidence of the disease in black, green and white polythene bags, the incidence of the disease was highest in the green and lowest in the black polythene bags.

6.0 CONTROL OF THE OIL PALM BLAST DISEASE BY CULTURAL PRACTICES

Blast is an important disease of oil palm seedlings in the nursery. It was, therefore, desirable to control the disease in order to minimise seedling losses. This section deals with the control of the disease by cultural practices.

6.1 Effect of watering on the control of the oil palm blast disease

In an earlier experiment in this study (Section 5.1), it was observed that the incidence of the blast disease was higher in the rainy than dry season. This suggests that water supply and soil temperature may have some effects on the disease. The aim of this experiment was to find out the effect of watering on the disease.

Extension work seedlings from the prenursery were planted in April in black polythene bags (20" x 15" layflat and 0·005" gauge) filled with non-sterilised topsoil from the wet season nursery at the NIFOR Main Station. Four hundred seedlings were planted singly in the polythene bags and the same number of seedlings from the prenursery was planted in the ground bed nursery for comparison. One half of the total number of seedlings planted in polythene bags and the same number planted in ground beds were supplied twice weekly with 2.5 pints and 4 pints of water per seedling respectively. The remaining seedlings were not supplied with water to serve as control. During this period, the seedlings received whatever natural precipitation

there was from dry season rain and dew. There were four replications for each treatment. Records of the blast disease incidence were taken from October of the year of planting to March of the following year. The percentage blast was calculated on the basis of the total number of seedlings planted. For the statistical analysis of the data, the percentage blast was transformed as earlier described (Section IV). The results of the statistical analysis are presented in Table 42.

Table 42

Losses due to the blast disease among oil palm seedlings supplied and not supplied with water

Treatments	Percentage blast (transformed)	
	Polythene bag planting	Ground bed planting
Watered	1·54*	2·18
Non-watered	2·26	1·83

* P = 0.05

The results of the experiment (Table 42) show an interesting difference between the seedlings in polythene bags and those in ground beds. In polythene bag planting, watering of oil palm seedlings significantly reduced (P = 0.05) the blast disease in comparison with non-watered plots. Conversely, watering appeared to aggravate the disease in ground bed planting. The

effect was, however, not statistically significant.

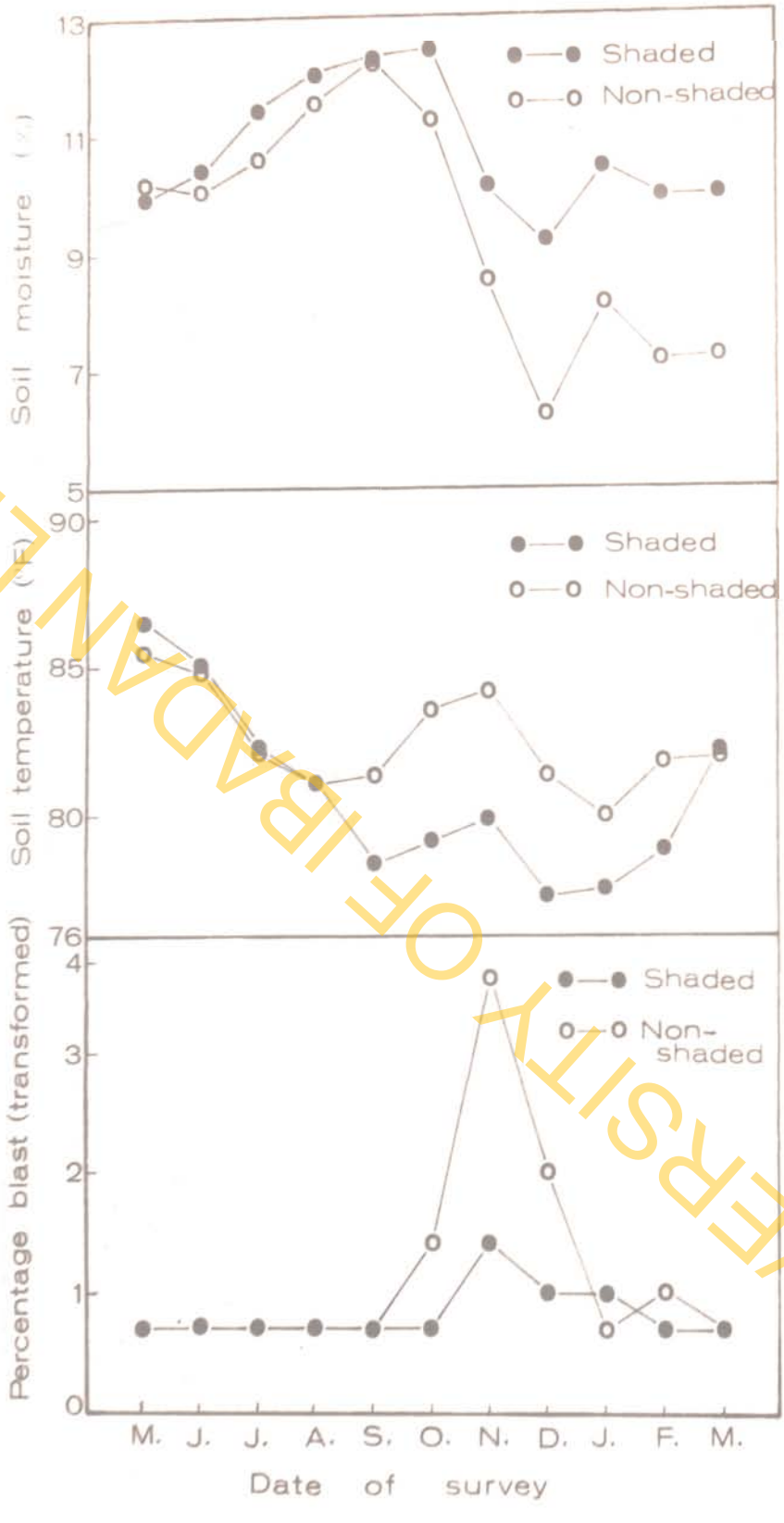
6.2 Effect of shade on soil temperature and soil moisture in relation to the control of the oil palm blast disease

The effect of watering on the control of the blast disease has already been investigated (Section 6·1). The aim of this experiment was to investigate the effect of shade on soil temperature and soil moisture in relation to the control of the blast disease.

The experiment comprised 16 plots each of 50 seedlings. Extension work seedlings from the prenursery were planted in eight ground bed plots in April while the remaining plots were planted with similar seedlings in black polythene bags (20" x 15" layflat and 0.005" gauge) filled with non-sterilised topsoil from the wet season nursery at the NIFOR Main Station. Four plots (replications) of polythene bag seedlings and the same number of plots of ground bed seedlings were shaded on the east-western sides and overhead at a height of 7 feet above the ground with palm leaves supported by a framework of sticks. The plots were shaded from September of the year of planting to February of the following year. The control plots were not shaded. Individual polythene bag and ground bed seedlings were supplied twice weekly with 2·5 pints and 4 pints of water respectively.

Thermometers calibrated in degrees fahrenheit were inserted vertically in the soil with their bulbs at a depth of 7 inches below ground level. A

Fig. 18. Effect of shade on soil temperature, soil moisture and the incidence of the oil palm blast disease in a ground bed nursery at the NIFOR Main Station.



thermometer was installed in each plot of ground bed seedlings and one in a polythene bag per plot of polythene bag seedlings. Temperature readings were taken daily at 1400 hours and the mean values were calculated separately for ground bed and polythene bag plantings. The mean temperature for each month was calculated from the means of daily readings. Soil temperature values were recorded from May of the year of planting to March of the following year.

Soil moisture content was determined gravimetrically. Cores of soil were taken from 9-12 inches zone with a tubular sampler. Two samples were taken at random from each plot and the total of eight samples per treatment were bulked to form a composite sample. Composite samples were taken thrice a month from May of the year of planting to March of the following year. The moisture content of each composite sample was determined on the day of collection. The mean value for each month was calculated.

Blast surveys were carried out at weekly intervals from May of the year of planting to March of the following year. The number of seedlings affected by the disease in a month was expressed as a percentage loss in relation to the total number of seedlings planted. For the statistical analysis of the data, the percentage blast was transformed as earlier reported (Section IV). The effects of shade on the blast disease, soil temperature and soil moisture in ground bed planting are shown in Table 43 and illustrated in Fig. 18.

Table 43

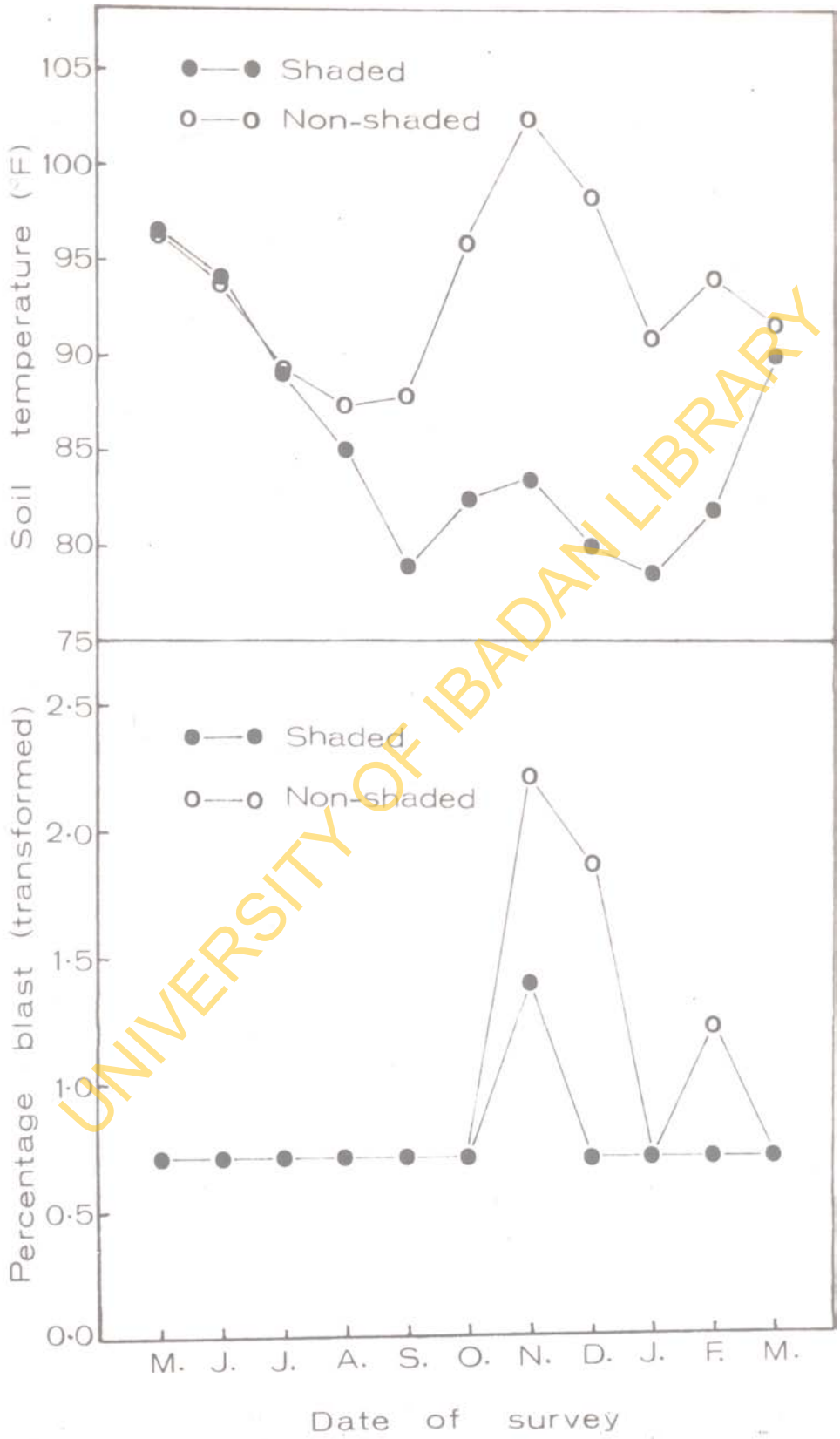
Effects of shade on soil temperature, soil moisture and the blast disease among seedlings planted in ground beds at the NIFOR Main Station

Month of determination	Percentage blast (transformed)		Soil temperature (°F)		Soil moisture (%)	
	Shaded	Non-shaded	Shaded	Non-shaded	Shaded	Non-shaded
May	0.7071	0.7071	86.6	85.4	9.9	10.2
June	0.7071	0.7071	85.1	84.7	10.4	10.1
July	0 7071	0.7071	82.4	82.1	11.4	10.6
August	0.7071	0.7071	81.1	81.0	12.0	11.5
September	0.7071	0.7071	78.4	81.3	12.3	12.2
October	0.7071	1.4142	79.2	83.5	12.4	11.2
Nocember	1.4142	2.6056	79.2	84.2	10.1	8.5
December	1.0000	2.0000	77.2	81.3	9.2	6.2
January	1.0000	0.7071	77.6	80.0	10.4	8.1
February	0.7071	1.0000	78.9	81.8	9.9	7.1
March	0.7071	0.7071	82.2	81.8	9.9	7.3

(a) Effect of shade on the control of the blast disease

Shade effectively reduced seedling losses due to the blast disease (Fig. 18). The correlation coefficient ($r = 0.8816$) between the blast disease in shaded and non-shaded plots was significant at the 0.1% level.

Fig. 19. Effect of shade on soil temperature and the incidence of the oil palm blast disease in a polythene bag nursery at the NIFOR Main Station.



(b) Effect of shade on soil temperature

From September to February when plots were shaded, there was a reduction in soil temperature (Fig. 18). There was, however, no statistically significant difference between soil temperature in shaded and non-shaded plots. There was also no significant correlation between the blast disease and soil temperature in shaded or non-shaded plots.

(c) Effect of shade on soil moisture

During the period of shade (September-February), soil moisture was conserved (Fig. 18). The difference in the soil moisture between shaded and non-shaded plots was not statistically significant.

There was a negative, but not statistically significant, correlation between the blast disease and soil moisture in shaded or non-shaded plots (Fig. 18).

The effects of shade on the blast disease and soil temperature in polythene bag planting are shown in Table 44 and illustrated in Fig. 19.

Table 44

Effects of shade on soil temperature and the blast disease among seedlings planted in polythene bags at the NIFOR Main Station

Month of determination	Percentage blast (transformed)		Soil temperature (°F)	
	Shaded	Non-shaded	Shaded	Non-shaded
May	0.7071	0.7071	96.6	96.4
June	0.7071	0.7071	94.5	94.0
July	0.7071	0.7071	89.2	89.5
August	0.7071	0.7071	85.1	87.5
September	0.7071	0.7071	79.1	88.0
October	0.7071	0.7071	82.5	96.0
November	1.4142	2.2361	83.4	102.5
December	0.7071	1.8708	80.1	98.5
January	0.7071	0.7071	78.6	91.1
February	0.7071	1.2247	81.9	94.1
March	0.7071	0.7071	90.1	91.8

(a) Effect of shade on the blast disease

Shade was again found to reduce seedling mortality due to the blast disease (Fig. 19). The correlation coefficient (r = 0.7462) between the blast disease in shaded and non-shaded plots was significant at the 0.1% level.

(b) Effect of shade on soil temperature

There was a reduction in soil temperature when plots were shaded from September to February (Fig. 19). The correlation coefficient between soil

temperature in shaded and non-shaded plots was, however, not statistically significant.

The correlation coefficient ($r = 0.775$) between the blast disease and soil temperature in non-shaded plots was significant at the 1% level. The correlation between the disease and soil temperature in shaded plots was not statistically significant.

6.3 Effect of shade on the control of the oil palm blast disease in non-cultivated forest soil

The blast disease occurred among oil palm seedlings planted in both cultivated and non-cultivated soils as recorded in section 5.4. The aim of this experiment was to carry out investigations into the control of the disease in non-cultivated forest soil.

Extension work seedlings of uniform size from the prenursery were planted in July in secondary forest soil at the NIFOR Main Station. Four hundred seedlings were planted in ground beds under the shade of secondary forest vegetation. The same number of seedlings was similarly planted in ground beds in another area of the secondary forest. The latter group of seedlings was not under forest canopy shade. All the seedlings were mulched with oil palm bunch refuse. Weeds were regularly pulled out but the seedlings were given no other cultural treatments. The late planting and lack of both watering and the application of fertilisers were designed to retard the growth

of the seedlings thus increasing their susceptibility to the blast disease. The seedlings were surveyed at weekly intervals for blast. The number of seedlings which succumbed to the disease was expressed as a percentage of the total number of seedlings planted. The results are given in Table 45 and illustrated in Fig. 20.

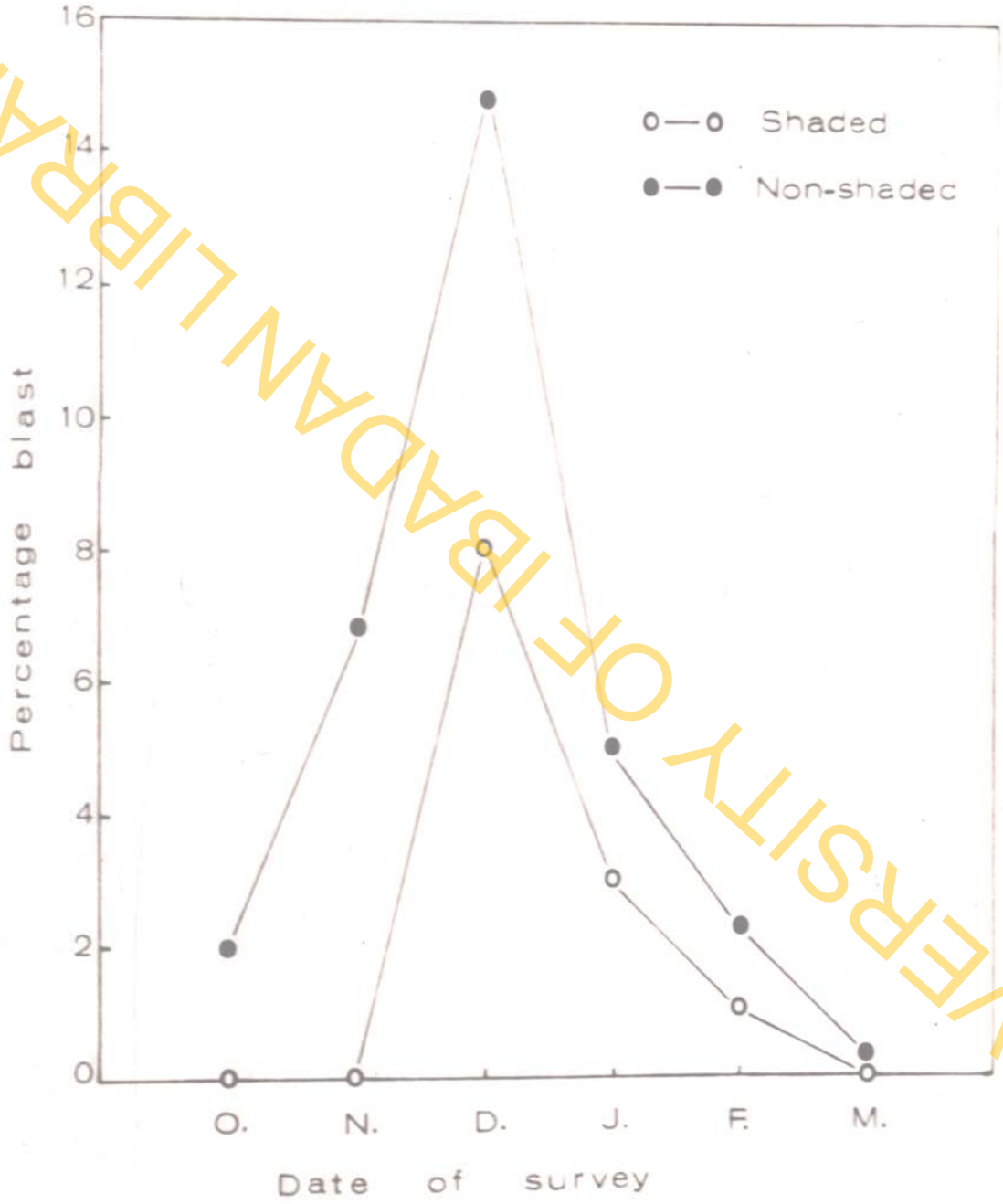
Table 45.

Losses due to the blast disease among shaded and non-shaded seedlings in non-cultivated forest soil.

Month of survey	Incidence of the blast disease (%)	
	Shaded seedlings	Non-shaded seedlings
October	0.0	2.0
November	0.0	6.8
December	8.0	14.8
January	3.0	5.0
February	1.0	2.3
March	0.0	0.3
Total	12.0	31.2

Seedling losses due to the blast disease were 2.5 times as high among non-shaded seedlings as those which were shaded. The months of November, December and January were the worst for the disease (Fig. 20).

Fig. 20. Incidence of the blast disease among oil palm seedlings planted in shaded, non-cultivated forest soil at the NIFOR Main Station.



6.4 The effect of a combination of different cultural practices on the control of the oil palm blast disease

The effects of polythene bag planting, ground bed planting, watering and shade on the oil palm blast disease have earlier been studied individually in this investigation. The effect of a combination of these factors on the incidence of the disease was investigated.

Eight treatments were compared in a randomised block design of four replications. There were 4 treatments in polythene bags and the same number of treatments in ground beds. Details of the treatments are given as follows:

T_1 = Polythene bag nursery, without shade or watering

T_2 = Polythene bag nursery, without shade, with watering

T_3 = Polythene bag nursery with shade, without watering

T_4 = Polythene bag nursery with shade and watering

T_5 = Ground bed nursery, without shade or watering

T_6 = Ground bed nursery, without shade, with watering

T_7 = Ground bed nursery, with shade, without watering

T_8 = Ground bed nursery, with shade and watering

Black polythene bags (20" x 15" layflat and 0.005" gauge) with twenty drainage holes each 0.25 inch-diameter were filled with non-sterilised topsoil from the wet season nursery at the NIFOR Main Station. Fifty bags

were arranged at $2\frac{1}{2}$ feet spacing in each plot. Extension work seedlings of uniform size from the pre-nursery were planted in April in the polythene bags and ground beds. Individual ground bed and polythene bag seedlings were supplied twice weekly with 4 pints and 2.5 pints of water respectively. Plots were shaded (as described earlier in section IV) from September of the year of planting to February of the following year. All the seedlings were given the usual cultural treatments. Blast disease records were taken at weekly intervals from October of the year of planting to March of the following year. For statistical analysis of the data, the percentage blast was transformed as reported under Materials and Methods (Section IV). The heights, number of leaves and percentage of transplantable seedlings were determined one year after nursery planting. The transplantable seedlings were seedlings big enough to be planted into the field where they were not affected by the blast disease. The results are presented in Table 46.

Table 46

Control of the blast disease by cultural practices in relation to seedling height, leaf production and percentage of transplantable seedlings in the nursery

Treatments	Percentage blast (transformed)	Mean seedling height(inches)	Mean number of leaves/palm	Transplantable seedlings (%)
T_1	2.26	46.75	11.65	77.50
T_2	1.54	51.12	12.40	87.50
T_3	1.03	50.77	10.92	91.50
T_4	0.93	50.15	10.87	89.00
T_5	1.83	67.77	12.82	84.00
T_6	2.18	69.37	13.30	79.00
T_7	0.78	65.60	12.00	97.50
T_8	0.96	63.62	10.67	92.50
Least significant differences:				
P = 0.05	0.558	5.88	1.11	8.32
P = 0.01	0.760	7.12	1.53	10.07
P = 0.001	1.026	10.80	2.06	15.28

(a) Control of the blast disease

Shading of nursery seedlings alone (T_3 and T_7) or together with watering (T_4 and T_8) was found to be the most effective method of reducing the incidence of the blast disease. This confirms an earlier result (Section 6.2). In polythene bag planting, shade with watering (T_4) was better than shade

without watering (T_3) in the control of the disease. Conversely, shade without watering (T_7) was found to be more effective than shade with watering (T_8) in the control of the disease in ground bed planting.

The incidence of the blast disease was lower in polythene bag than in ground bed plantings when the seedlings were supplied but not when they were not supplied with water.

In polythene bag planting, the combination of shade and watering (T_4) was significantly better ($P = 0.001$) than plots which were not shaded or watered (T_1) in the control of the blast disease. In ground bed planting, however, the combination of shade and watering (T_8) was only better than plots which were not shaded or watered at the 5% level of significance in the control of the disease.

(b) Heights of seedlings

Shade appeared to reduce seedling heights in comparison with non-shaded plots. Thus shaded ground bed seedlings (T_7 and T_8) were found to be shorter than non-shaded seedlings (T_5 and T_6). Similarly, shaded polythene bag seedlings (T_3 and T_4) were shorter than non-shaded seedlings (T_2 but not T_1).

Watered but non-shaded polythene bag seedlings (T_2) were taller than polythene bag seedlings which were not watered or shaded (T_1). Similarly, watered but non-shaded ground bed seedlings (T_6) were taller than seedlings

which were not watered or shaded (T_5).

Ground bed seedlings (T_5 - T_8) were significantly taller ($P = 0.001$) than polythene bag seedlings (T_1 - T_4).

(c) Production of leaves

Shade was found to decrease leaf production in comparison with non-shaded plots. Thus shaded polythene bag seedlings (T_3 and T_4) produced fewer leaves than non-shaded seedlings (T_1 and T_2). Similarly, shaded ground bed seedlings (T_7 and T_8) produced fewer leaves than non-shaded seedlings (T_5 and T_6).

Watering appeared to enhance leaf production in non-shaded but not in shaded plots. Thus polythene bag seedlings which were watered but not shaded (T_2) produced a larger number of leaves than seedlings which were not shaded or watered (T_1). A similar observation was made on ground bed seedlings.

Ground bed seedling (T_5 - T_8) produced a larger number of leaves than polythene bag seedlings (T_1 - T_4).

(d) Transplantable Seedlings

Shade appeared to increase the percentage of transplantable seedlings. The percentage of transplantable seedlings in shaded polythene planting (T_3 and T_4) was higher than in non-shaded plots (T_1 and T_2). Similarly, the percentage of transplantable seedlings was higher in shaded ground bed plots

(T_7 and T_8) than in non-shaded plots (T_5 and T_6).

Watering was found to increase the percentage of transplantable seedlings in non-shaded (but not in shaded) polythene bag planting. Thus the percentage of transplantable seedlings was higher in watered and non-shaded polythene bag planting (T_2) than in non-watered and non-shaded plots (T_1). Conversely, watering of ground bed seedlings (T_6 and T_8) was found to reduce the percentage of transplantable seedlings in comparison with non-watered plots (T_5 and T_7 respectively). The percentage of transplantable seedlings was higher in ground bed (T_5 - T_8) than in polythene bag planting (T_1 - T_4).

6.5 The relationship between the control of oil palm blast disease by cultural practices in the nursery and the subsequent establishment of the seedlings in the field.

In order to relate the control of the blast disease in the nursery to subsequent establishment of the young palms in the field, twenty palms from each of the 32 plots referred to in the last experiment (Section 6.4) were transplanted as described earlier (Section IV) into the field 14 months after nursery planting. The design of the experiment in the field was the same as that in the nursery. All the palms were given the usual cultural treatments in the field as described in section IV. The establishment of the young palms was determined 9 months after field planting. The height of the young palms and the number of fully-opened leaves produced by individual

palms were determined a week after field planting. The mean for each treatment was calculated. The determination was repeated 9 months after field planting. The mean increases in height and leaf production were determined by difference. The percentage of established palms was calculated on the basis of the number of young palms originally transplanted into the field. Established palms were those which continued to grow in the field after they had been transplanted from the nursery. The results are shown in Table 47.

Table 47

Leaf production, height and percentage of establishment 9 months after field planting from the combined shade and watering treatment experiment

Treatments	Established palms (%)	Mean increase in height/palm (inches)	Mean increase in no. of leaves/palm
T_1	95	21.700	9.850
T_2	99	16.575	10.900
T_3	100	17.050	9.800
T_4	97	19.300	9.075
T_5	97	5.750	7.400
T_6	100	4.500	7.350
T_7	97	7.700	8.300
T_8	100	11.800	8.875

Least significant differences:

P = 0.05		5.918	1.2778
P = 0.01	N.S.	8.055	1.7392
P = 0.001		10.866	2.3462

N.S. means not significant.

(a) Establishment of palms

The palms established satisfactorily in the field and were not affected by the blast disease. There were no significant differences between the treatments.

(b) Increase in the heights of the young palms

The smallest seedlings in the nursery as reported in section 6.4 produced the greatest increase in height in the field (T_1, T_4 and T_3). Conversely, the biggest seedlings in the nursery produced the least increase in height in the field (T_6, T_5 and T_7).

Shaded polythene bag seedlings in the nursery were found to produce a greater increase in height in the field (T_3 and T_4) than those which were not shaded (T_2 but T_1 excepted). Similarly, shaded ground bed seedlings in the nursery produced a greater increase in height in the field (T_7 and T_8) than those which were not shaded (T_5 and T_6).

Non-watered, non-shaded polythene bag seedlings in the nursery produced a larger increase in height in the field (T_1) than watered and non-shaded

seedlings (T_2). A similar observation was made on ground bed seedlings.

The combination of shade and watering in the nursery appeared to produce a greater increase in the height of polythene bag seedlings (T_4) and ground bed seedlings (T_8) in the field than non-watered, shaded polythene bag (T_3) and ground bed (T_7) seedlings respectively.

Polythene bag seedlings produced a greater increase in height in the field than ground bed seedlings. The difference between the height of polythene bag and ground bed seedlings (T_8 excepted) were significant at the 1% level.

(c) Increase in the number of leaves produced

Shaded, ground bed seedlings at the nursery stage produced more leaves at the field stage (T_7 and T_8) than non-shaded seedlings (T_5 and T_6).

There was no significant difference between the number of leaves produced by watered and non-watered ground bed or polythene bag seedlings.

There was no significant difference between the number of leaves produced by ground bed seedlings which were shaded and watered (T_8) and polythene bag seedlings (T_4).

Polythene bag seedlings (T_1 - T_4) produced more leaves in the field than ground bed seedlings (T_5 - T_8).

6.6 Effect of different periods of shade on seedling growth and the control of the oil palm blast disease

Although shade effectively reduced the incidence of the blast disease as described earlier, it also reduced the vigour of nursery seedlings (Section 6.4). Furthermore, the leaves of seedlings which were shaded from September to February were scorched by solar radiation and appeared to be predisposed to infection by weak fungal parasites such as Pestalotia sp. soon after the removal of the shade. It was, therefore, decided to investigate the effect of reducing the period of shade to a minimal level on seedling growth and the incidence of the blast disease.

In the experiment, ten treatments were compared in a randomised block design with four replications. Five treatments were applied to polythene bag seedlings and the same number of treatments was applied to ground bed seedlings. Shading of the seedlings was carried out at monthly intervals beginning from September to find out the month of the year when shading should commence. The seedlings were shaded for 3 months because earlier experiments reported in section 5.1 have shown that the blast disease was severe only in 2 - 3 months of the year. Details of the treatments are given as follows:

T_1 = Polythene bag nursery, shaded from September to November

T_2 = Polythene bag nursery, shaded from October to December

T_3 = Polythene bag nursery, shaded from November to January

T_4 = Polythene bag nursery, shaded from December to February

T_5 = Polythene bag nursery, shaded from September to February

T_6 = Ground bed nursery, shaded from September to November

T_7 = Ground bed nursery, shaded from October to December

T_8 = Ground bed nursery, shaded from November to January

T_9 = Ground bed nursery, shaded from December to February

T_{10} = Ground bed nursery, shaded from September to February

Black polythene bags (17" x 16" layflat and 0.005" gauge) with two drainage holes at the base were filled with non-sterilised topsoil and arranged at $2\frac{1}{2}$ feet spacing in the NIFOR 1971 wet season nursery. Fifty polythene bag were arranged in each plot. Extension work seedlings of uniform size from the prenursery were planted singly in the bags in July. The same type and number of seedlings were also planted in ground beds for comparison. Each plot was shaded on the east-western sides and overhead at a height of about 7 feet above the ground with palm leaves supported by a frame-work of sticks. Individual polythene bag and ground bed seedlings were supplied twice a week with 2.5 pints and 4 pints of water respectively. All the seedlings were given the usual cultural treatments. Blast incidence was

assessed at weekly intervals from October of the year of planting to March of the following year. Seedling loss due to the blast disease was expressed as a percentage of the original number of seedlings planted. The percentage blast was transformed as earlier described (Section IV) for the purpose of statistical analysis. Height, leaf production and percentage of transplantable seedlings based on the original number of seedlings planted were determined 10 months after nursery planting. The results are shown in Table 48.

Table 48

Reduction in the incidence of the blast disease by shading for different periods of the year in relation to seedling height, leaf production and percentage of transplantable seedlings

Treatments	Percentage blast (transformed)	Mean seedling height(inches)	Mean no. of leaves/ seedling	Transplantable seedlings (%)
T_1	1.22	33.55	11.3	78
T_2	0.91	31.85	10.1	76
T_3	1.39	34.25	10.4	71
T_4	1.39	39.27	10.8	64
T_5	0.78	35.50	10.4	78
T_6	1.72	38.90	11.2	73
T_7	1.03	37.95	12.3	84
T_8	1.52	35.87	11.2	66
T_9	2.11	39.27	10.6	49
T_{10}	0.78	39.05	10.5	69
Least significant differences:				
P = 0.05	0.408	3.778	1.13	
P = 0.01	0.511	5.108	-	
P = 0.001	0.734	-	-	

(a) Control of the blast disease

Shading of oil palm seedlings from September-February (T_5 and T_{10}) was significantly better (P = 0.05) than any of the other treatments in

reducing the incidence of the blast disease. The only exception was shading from October-December (T_2 and T_7) which was not statistically less effective than shading from September-February (T_5 and T_{10}). Shading from September-February (T_5 and T_{10}) was significantly superior ($P = 0.001$) to shading from December-February (T_9). Casualties due to the blast disease were found to be heavier among ground bed than polythene bag seedlings.

(b) Height of the seedlings

The tallest seedlings were found to be those which were shaded from December-February (T_4 and T_9). The shortest were polythene bag seedlings which were shaded from October-December (T_2). The difference between the tallest and shortest seedlings was significant at the 1% level. Ground bed seedlings were generally taller than polythene bag seedlings.

(c) Leaf production

Ground bed seedlings which were shaded from October-December (T_7) produced the largest number of leaves. The smallest number of leaves was produced by polythene bag seedlings which were shaded from October-December (T_2). The difference between the largest and smallest numbers of leaves was found to be significant at the 5% level.

The leaves of seedlings which were shaded from September-February (T_5 and T_{10}) were more severely scorched by solar radiation than any other group of seedlings.

(d) Transplantable seedlings

The highest percentage of transplantable seedlings was obtained in ground bed plots which were shaded from October-December (T_7). The lowest percentage was obtained in ground bed plots which were shaded from December-February (T_9).

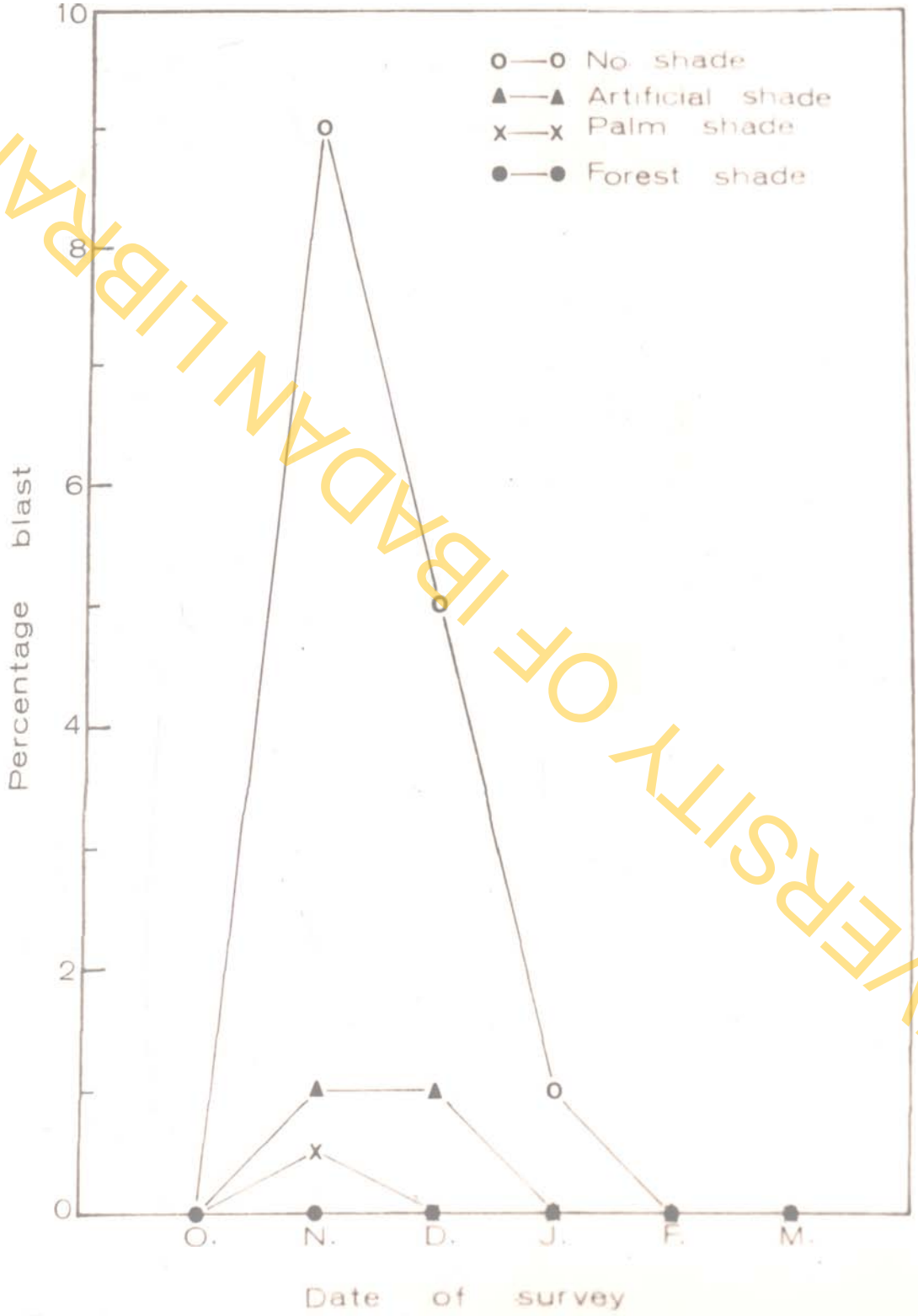
6.7 Effect of the types of shade on seedling growth and the control of the oil palm blast disease

The results of the experiments described earlier (Sections 6.2 and 6.4) have shown that artificial shade under pruned palm leaves effectively decreased the blast disease incidence. The leaves used in shading were pruned from mature palms which were actively producing fruit bunches. Such a practice is probably detrimental to yield. Furthermore, palm leaves are difficult to obtain in some parts of Nigeria. It was, therefore, decided to find alternative types of shade. Natural shade under secondary forest and under plantation palms were compared with artificial shade under pruned palm leaves. Natural shade has a high utility value because it is cheap, easy to adopt and found in most oil palm-growing areas of Nigeria.

Extension work seedlings from the prenursery were planted in July in black polythene bags (17" x 16" layflat and 0.005 gauge) filled with non-sterilised topsoil. The late planting was designed to enhance the incidence of the blast disease. The polythene bags were arranged in four plots, each

comprising 100 polythene bags. The first plot was shaded on the east-western sides and overhead at a height of about 7 feet above the ground with palm leaves supported by a framework of sticks (artificial shade). The second group of polythene bag seedlings was transferred from the nursery in October of the year of planting and arranged at $2\frac{1}{2}$ feet spacing under the shade of 15 year-old palms in field 14 at the NIFOR Main Station (palm shade). The third group of seedlings was similarly transferred and arranged under the shade of secondary forest vegetation in field 10 at the NIFOR Main Station (forest shade). The last group of seedlings remained unshaded in the nursery. All the seedlings were protected from rodents and other animal pests by a fence of wire netting. The seedlings were shaded for only four months (October to January). At the end of the shading period, the seedlings in fields 10 and 14 were returned to their original places in the nursery. The seedlings were individually supplied with 2.5 pints of water twice a week and given the usual cultural treatments. Assessment of the blast disease incidence was carried out at weekly intervals from October of the year of planting to March of the following year. Seedling mortality due to the disease was expressed as a percentage of the original number of seedlings planted. The results are illustrated in Fig. 21.

Fig. 21. Incidence of the oil palm blast disease among polythene bag seedlings placed under different types of shade at the NIFOR Main Station.



The result confirmed those of earlier experiments in this investigation that shade reduced the incidence of the blast disease. Natural shade under field planted palms or forest vegetation was more effective than artificial shade in reducing the disease incidence (Fig. 21). Forest shade in which none of the seedlings was affected by blast was the best. Although palm shade was effective, it exposed the seedlings to attack by the palm weevil, Rhyncophorus sp. The larvae of the insect attached 46% of the polythene bag seedlings under palm shade in December of the year of planting. The seedlings which recovered from the insect attack were stunted, malformed and unsuitable for planting into the field.

Seedling height, leaf production and percentage of transplantable seedlings were determined 10 months after nursery planting. The results are given in Table 49.

Table 49

Seedling height, leaf production and percentage of transplantable seedlings 10 months after nursery planting

Treatments	Mean height/seedling (inches)	Mean no. of leaves/seedling	Transplantable seedlings (%)
No shade	35.0	10.2	63
Artificial shade	37.7	11.9	86
Palm shade	25.8	7.5	20
Forest shade	34.2	8.4	80

(a) Heights of seedlings

Seedling placed under artificial shade were found to be the tallest. The shortest seedlings were those under the shade of 15-year old palms (palm shade). The heights of non-shaded and also seedlings under forest shade were between those of the tallest and the shortest.

(b) Leaf production

Seedlings placed under artificial shade were again found to produce the largest number of leaves. Seedlings under palm shade produced the lowest number of leaves. Leaf production by non-shaded and seedlings under forest shade was between those of the largest and lowest.

(c) Transplantable seedlings

The highest percentage of transplantable seedlings was obtained among seedlings placed under artificial shade. The lowest percentage was obtained among seedlings under palm shade. The percentages of transplantable seedlings among non-shaded and seedlings placed in forest shade were between those of the highest and the lowest.

6.8 Summary of results on the control of the oil palm blast disease by cultural practices

Shading of polythene bag or ground bed seedlings in the dry season particularly from October-December was found to be the most satisfactory method of reducing the incidence of the oil palm blast disease and producing

suitable seedlings for field planting. Natural shade under secondary forest vegetation was found to be more effective than artificial shade under pruned palm leaves in reducing the disease incidence. Palm shade, though effective, exposed the seedlings to severe attack by the larvae of the palm weevil, Rhyncophorus sp., which rendered most of the seedlings unsuitable for field planting.

Watering was found to reduce the blast incidence in polythene bag planting but appeared to aggravate the disease in ground bed seedlings. Seedling mortality due to the blast disease was lower in polythene bag than in ground bed plantings.

Seedlings which were transplanted from the nursery were found to establish satisfactorily in the field. Furthermore, they were not affected by the blast disease in the field. Polythene bag seedlings were found to establish faster and to grow more vigorously than ground bed seedlings.

7.0 CHEMICAL CONTROL OF THE OIL PALM BLAST DISEASE

Experiments carried out by earlier workers at the NIFOR Main Station on the chemical control of the blast disease were generally unsuccessful (Anon., 1953; Anon., 1954; Anon., 1963). This may be attributed to inadequate knowledge of the nature and cause of the disease because the workers wrongly assumed that the disease was caused by nematodes. The present investigation has shown that the blast disease is caused by the co-infection of oil palm seedlings by P. splendens and R. lamellifera. In the light of this finding, it was decided to carry out more experiments on the chemical control of the disease.

7.1 Chemical control of the oil palm blast disease in the nursery.

In this investigation, laboratory screening of fungicides against the causal organisms of the blast disease was first undertaken with the aim of selecting only the promising chemicals before taking up the time-consuming and costly nursery trials on the control of the blast disease. The results of the in vitro studies have been recorded earlier (Sections 4.6, 4.7, 4.14 and 4.15).

In the nursery experiment, eight treatments were compared in a randomised block design with four replications. Three selected fungicides (PCNB, Fernasan and Benlate) were compared with shade which was found in earlier experiments in this investigation (Section 6.2) to be the most effective

cultural method of reducing the incidence of the blast disease. Details of the treatments are given as follows:

T_1 = shade without fungicide

T_2 = PCNB without shade

T_3 = Fernasan without shade

T_4 = Benlate without shade

T_5 = PCNB with shade

T_6 = Fernasan with shade

T_7 = Benlate with shade

T_8 = Control (no shade or fungicide)

Black polythene bags (20" x 15" layflat and 0.005") with twenty drainage holes each 0.25 inch-diameter were filled with topsoil which had been partially sterilised by baking in a simple steel framework over an open fire. The bags were arranged at the rate of 50 per plot and at $2\frac{1}{2}$ feet spacing in the wet season nursery at the NIFOR Main Station. Uniform seedlings from the prenursery were planted in them in May. All the seedlings were given the usual cultural treatments. Each seedling was supplied with 2.5 pints of water twice a week. The chemicals were suspended separately in known volumes of water and 2.5 pints of the suspension were applied to the soil in each bag. Thus the soil (based on oven dry weight) in each polythene bag received the active ingredient weight equivalent to 100 p.p.m. of PCNB, 100

p.p.m. of Fernasan or 20 p.p.m. of Benlate. The chemical treatments were applied three times at monthly intervals i.e. October, November and December. The plots were shaded on the east-western sides and overhead at about 7 feet above the ground with palm leaves supported by a framework of sticks in the treatments requiring shade. The seedlings were shaded from September of the year of planting to February of the following year. Assessment of the blast disease was carried out at weekly intervals from September of the year of planting to March of the following year. The number of seedlings affected by blast was expressed as a percentage of the total number of seedlings planted per treatment. The percentage blast was transformed as earlier reported (Section IV) for the purpose of statistical analysis. The mean seedling height, number of leaves produced and percentage of transplantable seedlings were determined one year after nursery planting. The results are presented in Table 50.

Table 50.

Chemical control of the blast disease in relation to seedling height, leaf production and percentage of transplantable seedlings in the nursery.

Treatments	Percentage blast (transformed)	Mean seedling height (inches)	Mean no. of leaves per palm	Transplantable seedlings (%)
T_1	0.71	51.1	11.4	99.5
T_2	1.26	47.8	12.3	91.0
T_3	0.83	46.9	12.5	92.0
T_4	0.98	46.6	13.7	87.5
T_5	0.71	51.3	11.9	97.0
T_6	0.85	50.9	11.8	95.0
T_7	0.71	53.8	11.4	99.0
T_8	1.15	49.2	12.6	87.0
Least significant differences:				
P = 0.05	0.25	3.05	0.42	5.76
P = 0.01	0.31	4.15	0.57	7.84
P = 0.001	0.41	5.60	0.76	10.58

(a) Chemical control of the blast disease

Chemical treatments (T_2 - T_4) controlled the blast disease in comparison with untreated plots (T_8). The only exception was PCNB (T_2) which was found to aggravate the disease in comparison with untreated plots (T_8).

None of the chemical treatments (T_2, T_3 and T_4 representing PCNB Fernasan and Benlate respectively) was, however, as effective as shade alone

(T_1) in the control of the blast disease. Shade had earlier been reported (Section 6.2) to be the most effective cultural method of reducing the incidence of the disease.

The combination of chemical and shade (T_5 - T_7) was found to be more effective than chemical treatments alone (T_2 - T_4) in the control of the blast disease. The only exception was Fernasan with shade (T_6), which was found to be less effective than Fernasan alone (T_3) in the control of the disease.

(b) Mean height of seedlings

Seedlings treated with chemicals (T_2 - T_4) were found to be shorter than untreated (control) seedlings (T_8). Thus it appeared that the chemicals reduced the height of oil palm seedlings.

Seedlings treated with the combination of chemical and shade (T_5 - T_7) were significantly taller than those treated with chemicals alone (T_2 - T_4) at the 5% level.

(c) Leaf production

Leaf production by seedlings treated with chemicals (T_2 - T_4) was much the same as untreated seedlings (T_8). The only exception was chemical treatment with Benlate (T_4) which produced a significantly greater number of leaves ($P = 0.001$) than any other treatment.

Seedlings treated with the combination of chemical and shade (T_5 - T_7) produced a significantly fewer number of leaves than those treated with chemieals alone (T_2 - T_4). The former group of seedlings, however, produced a slightly greater number of leaves than seedlings which were shaded alone (T_1).

Non-shaded seedlings (T_8) produced a larger number of leaves than shaded seedlings (T_1). The difference between the number of leaves produced was statistically significant at the 0.1% level.

(d) Transplantable seedlings

Chemical treatments alone (T_2 - T_4) produced a higher percentage of transplantable seedlings than untreated seedlings (T_8).

Chemical treatments and shade (T_5 - T_7) produced a higher percentage of transplantable seedlings than chemical treatments alone (T_2 - T_4). The former group of seedlings (T_5 - T_4), however, produced a lower percentage of transplantable seedlings than shade without chemical treatment (T_1).

The percentage of transplantable seedlings was significantly higher ($P = 0.001$) in plots shaded alone (T_1) than in non-shaded plots (T_8).

7.2 The relationship between the chemical control of the oil palm blast disease in the nursery and the subsequent establishment of the seedlings in the field.

In order to relate the control of the blast disease in the nursery to the subsequent establishment of the young palms in the field, twenty palms from each of the 32 plots referred to in section 7.1 were transplanted as described earlier (Section IV) to the field as Experiment 27-8 14 months after nursery planting. The experimental design in the field was the same as that in the nursery. All the palms were given the usual cultural field treatments. The height, number of fully-opened leaves produced by individual palms were determined a week and also 9 months after field planting. The mean increases in height and leaf production were calculated. The percentage of established palms based on the number of palms planted into the field was also calculated. Established palms were those which continued normal growth in the field after they had been transplanted from the nursery. The results are presented in Table 51.

Table 51

Leaf production, height and percentage of establishment 9 months after field planting from shade and chemical treatment experiment.

Treatments	Established palms (%)	Mean increase in height/palm (inches)	Mean increase in no. of leaves/palm
T_1	100	20.6	9.625
T_2	100	21.4	9.500
T_3	98.7	22.8	9.325
T_4	98.7	22.7	10.150
T_5	100	20.5	10.575
T_6	100	19.2	9.325
T_7	100	17.1	8.750
T_8	100	19.4	10.100
Least significant differences:			
P = 0.05			1.081
P = 0.01	N.S.	N.S.	1.471
P = 0.001			

N.S. means not significant

(a) Established palms

The palms established satisfactorily in the field and were not affected by the blast disease. There were no significant differences between the treatments.

(b) Increase in height of seedlings

The mean increase in height of seedlings treated at the nursery stage with chemicals alone (T_2 - T_4) was greater than that of control seedlings (T_8). There were, however, no statistically significant differences between the treatments.

(c) Increase in the number of leaves produced

There were no statistically significant differences in leaf production between seedlings treated, at the nursery stage, with chemicals (T_2 - T_4) and control seedlings (T_8). There were also no significant differences in leaf production between seedlings treated, at the nursery stage, with a combination of chemical and shade (T_5 - T_7) and those treated with chemicals alone (T_2 - T_4). The only exception was Benlate without shade (T_4) which produced a significantly greater number of leaves ($P = 0.05$) than the combination of Benlate and shade (T_7).

7.3 Summary of results on the chemical control of the oil palm blast disease

Three chemicals (PCNB, Fernasan and Benlate) were tested for their effect on the incidence of the blast disease and the growth of oil palm seedlings. Fernasan and Benlate were found to control blast while PCNB aggravated the disease. None of the chemicals was, however, found to be as effective as shade alone in reducing the incidence of the disease.

The three chemicals tested appeared to reduce the height of oil palm seedlings. Benlate was, however, found to enhance leaf production. The chemicals did not produce undesirable side effects on the seedlings in the nursery or field. The seedlings treated with the chemicals in the nursery were found to establish satisfactorily in the field.

8.0 TESTING OF CERTAIN VARIETIES OF THE OIL PALM FOR POSSIBLE RESISTANCE TO THE BLAST DISEASE

Selection of oil palm varieties for disease resistance was not, unfortunately, incorporated with the NIFOR oil palm breeding programme which started in 1959. Because of this, the extension work seeds produced by the Institute and distributed to oil palm planters and farmers within and outside Nigeria are not screened for resistance to the blast disease. The aim of this section was, therefore, to test some varieties of the oil palm produced at NIFOR and some seeds of grove palms for resistance to the blast disease. The results of the tests are reported under separate subheadings.

8.1 Testing of grove palm and extension work seedlings for resistance to the blast disease.

It appears that no previous investigation has been carried out into the incidence of the blast disease among grove palm seedlings. In this experiment, the incidence of the disease among grove palms was compared with that among extension work seedlings.

Seeds of grove palms were collected from a secondary forest (Field 18) at the NIFOR Main Station. The seeds were germinated by the usual dry heat treatment method described earlier (Section IV). The sprouted seeds were planted into a prenursery. Five hundred of the resulting seedlings were transplanted into the wet season nursery at the NIFOR Main Station in May.

The same number of extension work seedlings of the same age was similarly planted in ground beds for comparison. All the seedlings were given the usual cultural treatments. Blast disease incidence was assessed at weekly intervals from October of the year of planting to March of the following year. The number of seedlings affected by blast was expressed as a percentage of the original number of seedlings planted. The results are presented in Table 52 and illustrated in Fig. 22.

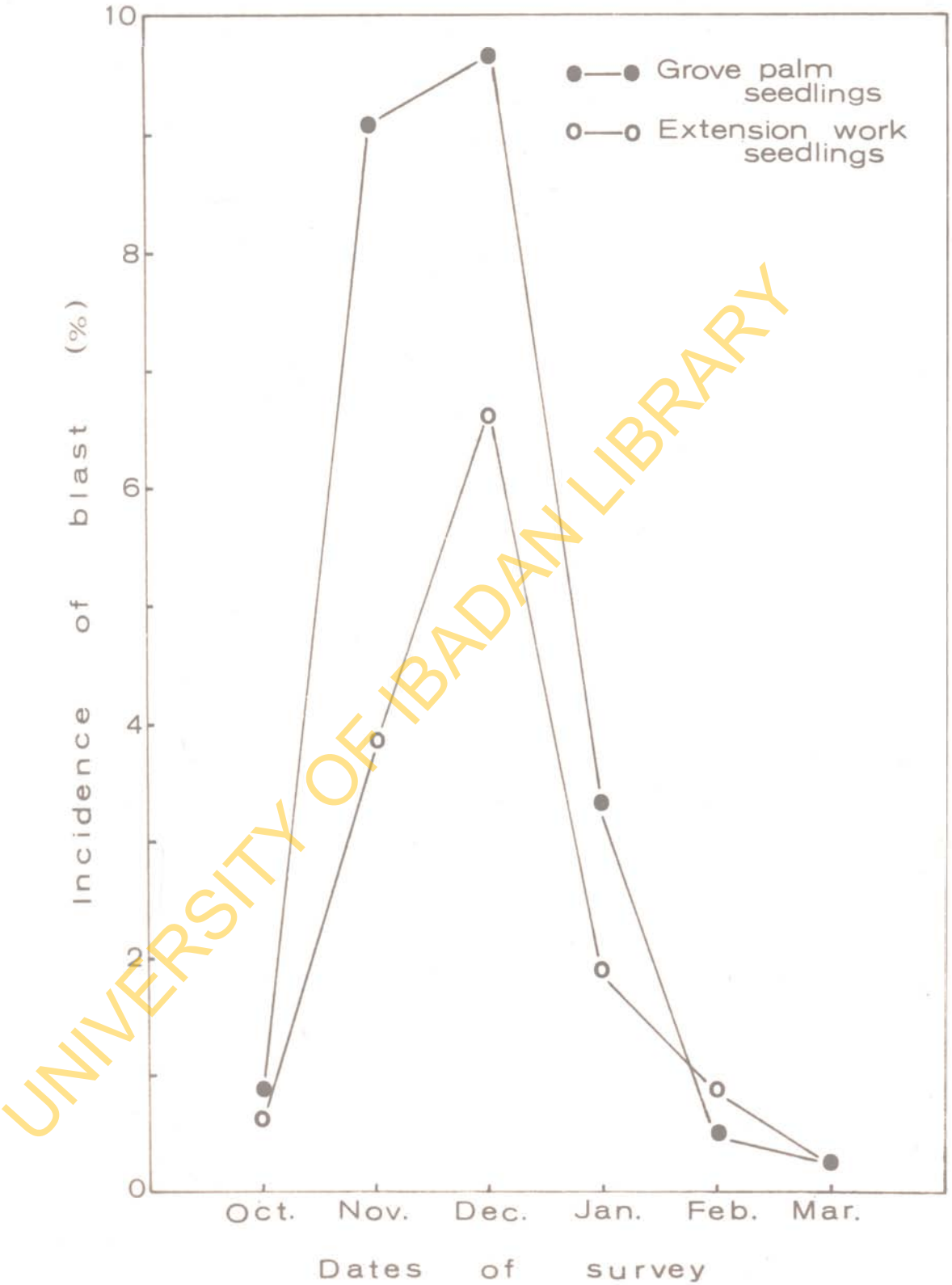
Table 52

Percentage losses due to the blast disease among grove palm and extension work seedlings

Month of survey	Incidence of the blast disease (%)	
	Grove palm seedlings	Extension work seedlings
October	0.8	0.6
November	9.0	3.8
December	9.6	6.6
January	3.2	1.8
February	0.4	0.8
March	0.2	0.2
Total	23.2	13.8

The trends of development of the disease were similar in both plantings. The incidence of the disease increased progressively from October to a peak in December. Thereafter the incidence of the disease decreased sharply and

Fig. 22. Incidence of the oil palm blast disease among grove palm and extension work seedlings planted in a wet season nursery at the NIFOR Main Station.



reached the minimal level in March (Fig. 22). The overall losses due to the blast disease among grove palm and extension work seedlings were 23.2% and 13.8% respectively. These results suggest that extension work seedlings are more resistant to the blast disease than grove palm seedlings.

8.2 Testing of different crosses of the oil palm for resistance to the blast disease.

NIFOR produces improved seeds by the controlled pollination of selected dura variety with pollen from pisifera variety of oil palm as described earlier (Section IV). These improved hybrid seeds, otherwise known as extension work seeds give rise, on germination, to tenera variety of palms. In addition to the dura x pisifera (D x P) cross normally produced for extension work, other crosses such as dura x dura (D x D), dura x tenera (D x T) and tenera x tenera (T x T) are also made at the NIFOR Main Station. The incidence of blast was compared among these crosses of palm as follows:

Seedlings obtained from each cross were planted in ground beds in the NIFOR wet season nurseries. The seedlings were given the usual cultural treatments. The incidence of the blast disease was assessed at weekly interval from October to March. Seedling losses due to the disease are given in Table 53.

Table 53

Seedling losses due to the blast disease among different crosses of oil palm

Types of cross	Incidence of the blast disease (%)		
	1st year	2nd year	Mean for 2 years
Dura x pisifera	14.1	5.9	10.0
Tenera x tenera	21.5	7.4	14.5
Dura x tenera	29.9	12.6	21.3
Dura x dura	34.5	37.9	36.2

Oil palm crosses showed differences in resistance to the blast disease. Dura x pisifera was found to be the most resistant while dura x dura was the least resistant cross to the disease.

8.3 Summary of results on the testing of certain varieties of the oil palm for possible resistance to the blast disease.

Extension work seedlings were found to be more resistant to the oil palm blast disease than grove palm seedlings.

Four types of cross were tested for resistance to the blast disease. Dura x pisifera (extension work seeds) was found to be the most resistant while dura x dura was the most susceptible cross to the disease.

SECTION VI

DISCUSSION

The series of investigations in this study showed that the oil palm blast disease occurred in all the nine nurseries and four nurseries surveyed for the disease in the Western State and the Mid-Western State of Nigeria respectively. The disease occurred between October and March and reached the highest incidence in November and December. The survey also revealed that seedling mortality due to the blast disease varied from year to year. At the NIFOR Main Station, for example, the annual seedling losses due to the blast disease were found during this investigation to vary between 8.6% and 16.8% among extension work seedlings planted into the nursery in April at the beginning of the rainy season in Nigeria. Higher seedling losses due to the disease were recorded among seedlings planted in June, July or August.

In addition to the oil palm, other palms of ornamental and economic importance were also found to be affected by the blast disease. Among such palms, Aiphanes acanthophylla (Mart.) Burret., Areca catechu Linn., Areca lynn, Chrysalidocarpus lutescens H. Wendl., Ptychosperma elegans Blume., Ptychosperma macarthurii H. Wendl. and Roystonea regia (H.B.K.) Cook were found to be highly susceptible to the disease.

The most frequently isolated fungi from infected roots of oil palm seedlings affected by the blast disease were Pythium splendens Braun

(Accession No. IMI 149554) and Rhizoctonia lamellifera Small (Accession No. IMI 149556). Both fungi were shown in inoculation experiments to be the causal organisms of the oil palm blast disease.

Ecological studies on blast pathogens showed that the infective propagules of the pathogens were widely distributed in soil and their recovery values from soil (determined by the root baiting technique) were found to be affected by cultural practices and soil environmental factors.

Linear growth, sporangial production and germination in P. splendens were found to be most satisfactory on Czapek-dox agar, cassava-dextrose agar and quaker-oats agar at a constant temperature of 30°C. Linear growth, sclerotial production and germination in R. lamellifera were best on potato-dextrose agar and V8-juice agar at a temperature of 30°C. Sclerotial longevity in R. lamellifera was highest at a relative humidity value of 95% and a low temperature of 20°C.

Various cultural factors including planting date, soil temperature, soil moisture and soil type, cropping history and planting containers were also found to affect the occurrence and level of incidence of the blast disease.

Shading of nursery seedlings from October to December was found to be the most effective cultural method of reducing the incidence of the blast disease. Soil treatment with Fernasan and Benlate was found to control the disease while soil treatment with PCNB appeared to aggravate the disease.

Extension work seedlings were found to be more resistant to the blast disease than grove palm seedlings.

These and other results will now be discussed in detail.

The results of the surveys which showed that the blast disease was widespread in oil palm nurseries throughout the Western and Mid-Western States of Nigeria (Tables 1 and 2) are similar to the observations of Robertson (1959a) who reported that the blast disease occurred in all oil palm nurseries throughout Nigeria. Surveys of the disease were, however, not carried out in other oil palm-growing States of Nigeria (notably South-Eastern, East-Central and Rivers States) because of the Nigerian civil war (1967-1969).

The incidence of the blast disease was found to vary between 8.6% and 16.8% during the 5 years of observation on extension work seedlings planted into nurseries at the NIFOR Main Station (Table 1). These losses are lower than the mortality of up to 20% which had earlier been recorded in nurseries at the WAIFOR (now NIFOR) Main Station (Robertson, 1959a). An annual loss of 8-10% was reported by Robertson (1959a) to be the seedling mortality in Nigerian nurseries between 1949 and 1959. Higher seedling losses of more than 50% have been reported in the Ivory Coast and Cameroun (Robertson, 1959a), about 80% in the Ivory Coast (Bachy, 1958) and 20-25% in an estate nursery in Malaysia (Turner, 1965; Turner and Bull, 1967). Blast is, therefore, considered to be a disease of great economic importance because of the

heavy losses among oil palm seedlings.

Apart from the oil palm, other palms of ornamental and economic importance were also observed to be affected by the blast disease in nurseries at the NIFOR Main Station (Table 3). Some of the palms such as Aiphanes acanthophylla, Areca catechu, Areca lynn, Chrysalidocarpus lutescens, Ptychosperma elegans, Ptychosperma macarthurii and Roystonea regia with a seedling mortality higher than 50% appeared to be more susceptible to the blast disease than the oil palm itself (Table 3). Seedlings of Caryota mitis Lour., Coccothrinax argentea K. Sch., Phoenix dactylifera Linn. Copernicia prunifera Mart. and Sabal umbraculifera Mart. were only slightly or not affected by the blast disease. The reason for this is, however, not clearly understood. Robertson et al. (1968) had previously recorded the incidence of the blast disease among seedlings of Areca catechu, Corozo oleifera (H.B.K.) Bailey, Euterpe Cocos nucifera Linn. and Roystonea oleracea (Mart.) Cook. This investigation has confirmed their observation. The incidence of the disease among seedlings of Aiphanes acanthophylla, Areca lynn, Chrysalidocarpus lutescens, Copernicia prunifera, Ptychosperma elegans and Ptychosperma macarthurii which was reported in this investigation are new records. It is important to emphasise the role of these palms as reservoirs of the disease, especially in view of the high level of incidence of the disease on some of them.

There was no difficulty encountered in the isolation of R. lamellifera from infected roots or sclerotia found in infected root tissues. The isolation of P. splendens from infected roots of oil palm was, however, problematic. That this has been a long-standing problem is shown by the fact that it took 15 years from the time the oil palm blast disease was first observed and described by Trueblood in 1944, cited by Robertson (1959a), to the time the cause of the disease was conclusively determined by Robertson (1959b). The isolation of the fungus by plating roots showing primary infection directly on agar culture media or by the washing technique adopted by Robertson (1959a and 1959b) was found to be unsatisfactory. The most satisfactory method of isolating the fungus was found to be by the direct plating of infected root segments on cassava-dextrose agar. This special medium had previously been used in the cultural studies on Phytophthora palmivora (Butl.) Butl., the cause of the black-pod disease of cocoa (Weststeijn, 1963; Akinrefon, 1967) and on some other fungi (Maduewesi and Nwauzo, 1966).

The results of the pathogenicity tests (Tables 8 and 9) confirm the findings of Robertson (1959b) that the oil palm blast disease is caused by both Pythium splendens Braun and Rhizoctonia lamellifera Small. In this study, P. splendens was found to be strongly parasitic while R. lamellifera appeared to be weakly parasitic on oil palm seedlings. This may be attributed to the observation made by Robertson (1959b) that Pythium sp. was capable while

R. lamellifera was incapable of penetrating healthy undamaged roots of oil palm seedlings. Infection was more severe on seedlings inoculated with P. splendens than on those inoculated with the mixture of P. splendens and R. lamellifera. This contradicts the result obtained by Robertson (1959a) who found that seedlings inoculated with the mixture of Pythium sp. and R. lamellifera developed the blast disease symptoms more rapidly than seedlings inoculated with Pythium sp. alone. A possible explanation for this was offered by the work of Robertson (1959a) who observed that the development of the blast disease on artificially-inoculated seedlings was dependent upon the growth stage of the seedlings because prenursery seedlings were used for the pathogenicity test in this investigation while Robertson used older seedlings removed from the nursery. The mixed inoculum, unlike the Pythium inoculum alone, induced symptoms which were typical of the blast disease. The production of sclerotia in localised root lesions by R. lamellifera suggests that the experimental conditions were probably unfavourable for the growth of and infection by the fungus.

Ecological studies on P. splendens showed that the fungus was widely distributed in both forest and cultivated, arable soils in south-western Nigeria (Table 14). The fungus was also isolated from diseased oil palm seedlings in all the four nurseries in the Mid-Western State and four out of nine nurseries

surveyed for the disease in the Western State of Nigeria. The observation that P. splendens was not isolated from diseased oil palm seedlings in five of the nurseries during only one survey in 1967 cannot be taken as conclusive evidence that the fungus was absent from those nurseries. R. lamellifera, on the other hand, was found to be associated with diseased oil palm seedlings in almost all the nurseries surveyed for the blast disease in south-western Nigeria (Table 7). The occurrence of the blast disease in all the oil palm nurseries which were surveyed for the disease in south-western Nigeria demonstrates that the disease and its causal organisms (P. splendens and R. lamellifera)are widely distributed in south-western Nigeria. This observation is similar to the reports of Robertson (1959a), Garrett (1960 and 1970), Waterhouse and Waterston (1966), Turner and Bull (1967) that Pythium and Rhizoctonia species are widely distributed in tropical and sub-tropical areas of the world.

The isolation of P. splendens by baiting with discs of live oil palm roots showed that the fungus was unevenly distributed in soil. The recovery value of the fungus from cultivated nursery soil at the NIFOR Main Station was found to be highest at the ploughline depth of 9 inches (Fig. 2). Since dry atmosphere was found to reduce the viability of Pythium propagules in soil (Tables 12 and 13), many of those brought to or near the surface by ploughing and other

cultural operations would be expected to lose their viability. Thies and Patton (1970) found a similar gradient of increasing population with soil depth to the ploughline for the microsclerotia of Cylindrocladium scoparium.

Several factors including soil temperature, soil moisture, soil cultivation and organic manuring were found to affect the recovery of P. splendens from soil. Menzies (1970) observed that the effects of temperature on root diseases have been well studied but its direct effect on pathogen population has seldom been determined. This is probably true also for soil moisture. In this investigation, the recovery value of P. splendens as determined by the root baiting technique was found to be high in July-October which corresponded with the highest soil moisture. The recovery value of the fungus was low in November-January when soil moisture was also low (Fig. 3). The variation in soil temperature during this period was not high. Differences in the recovery of P. splendens from soil were, therefore, not likely to be affected by changes in soil temperature. This, probably is an over-simplification of a relationship involving a complex ecosystem in which the roles of several other factors and microorganisms may also be important but there is a positive indication of the importance of soil moisture. In addition to soil environmental factors, parasitism of P. splendens by R. lamellifera (Robertson, 1959b) and P. splendens by R. solani (Butler, 1957)

may cause a reduction in the inoculum density of P. splendens during the dry season. Okaisabor (1968) found that P. palmivora (the cause of the black-pod disease of cocoa) was commonly parasitised by Botryodiplodia theobromae Pat. and observed that in spite of this parasitism, the incidence of the black-pod disease was high in many cocoa farms in Nigeria. Fungal necrosis or lysis by antibiotic - producing bacteria reported by Huber and Andersen (1966) on Fusarium solani f. phaseoli and Rhizoctonia solani may also play an important role in the seasonal variation in the recovery value of P. splendens from soil. Such bacterial activities will presumably be more intense in the rainy than dry season but since Pythium thrives better in high than low soil moisture (Garrett, 1960; Waterhouse, 1968 and Wheeler, 1969) the recovery value of the fungus would be comparatively higher in the rainy than dry season. Nonetheless, a similar seasonal variation in the recovery value of Pythium from soil has been reported by McLaughlin (1947) who found that a combination of high soil temperature with low moisture generally resulted in a reduction in the percentage of Pythium isolates. The increase observed in this study in the recovery value of P. splendens with soil cultivation and organic manuring (Tables 14 and 15) is consistent with the result obtained by Chu (1966) and is probably attributable to the "high competitive saprophytic ability" of the fungus in colonising organic matter (Garrett, 1960 and 1970). Similar results were obtained by Watson (1970a) in studies on P. ultimum Trow. Watanabe in 1969,

cited by Snyder (1970), also found that cultural practices greatly influenced the survival and population increases of Macrophomina phaseoli (Maub.) Ashby. Because of practical difficulties, organic manure is seldom uniformly ploughed into nursery soils. Pockets of high organic matter in such circumstances would constitute loci of high Pythium density and oil palm seedlings which are inadvertently planted in such pockets would be exposed to a greater inoculum potential than seedlings planted in adjacent soil. It is possible for an oil palm seedling to be affected by the blast disease while the adjacent seedling of the same age and variety remained healthy. This was frequently observed in oil palm nurseries during this study. Such local variation in the recovery value of Pythium from soil were demonstrated in this investigation by the recovery value of the fungus in soil around the roots of diseased oil palm seedlings being 4 times higher than that around the roots of healthy seedlings (Table 16). This result contrasts with that obtained by Nash and Snyder (1962) who found that the distribution of the bean root rot (Fusarium sp.) was uniform in cultivated fields at a plow depth of 6-8 inches.

The literature on the physiology of P. splendens and R. lamellifera is very meagre. The greatest linear growth and sporangial production in P. splendens were obtained in Czapek-dox agar (Table 17). The highest percentage germination of sporangia was obtained in quaker-oat agar. This shows that

the best medium for growth was also the best for sporangial production but not necessarily the best for sporangial germination. The most suitable medium for the linear growth, sporangial production and germination in P. splendens was found to be Czapek-dox agar. Other media such as cassava-dextrose agar and potato-dextrose agar were also found to be suitable for the growth, sporangial production and germination in the fungus. Similar results were obtained by Braun (1925) who found that P. splendens produced abundant conidia (synonymous with sporangia) in 16 artificial media including oat-meal agar, corn-meal agar and potato-dextrose agar. He concluded that the richer media induced a more rapid and higher percentage germination of sporangia and also a large number of germ tubes per sporangium than the poor media.

In R. lamellifera the greatest growth was obtained on cassava-dextrose agar and potato-dextrose agar in which the fungus produced a mycelial diameter of 7.6 cm within 3 days at 25°C (Table 22). The most suitable medium for sclerotial production in the fungus was found to be V8-juice agar while sclerotial germination was best in V8-juice agar and potato-dextrose agar. These results suggest that the best medium for growth was also the best for sclerotial germination but not necessarily the best for sclerotial production. The best medium for the growth, sclerotial production and

germination in R. lamellifera was found to be V8-juice agar. Other media such as corn-meal agar and quaker-oat agar were also found to be suitable for growth, sclerotial production and germination in the fungus. Hopkins (1933) reported that the mycelial growth rate in R. lamellifera in corn-meal agar was in the range of 4.0-8.5 cm within 3 days of incubation at 25°C. The comparative growth rate of the isolate from oil palm obtained in this investigation was 5.1 cm at the same temperature. The medium which supported the least growth in the fungus was found to be soluble starch agar. A similar result was reported by Hopkins (1933) who found that highly concentrated starch media inhibited the growth in R. lamellifera. These results indicated that the medium which was found to be the most satisfactory for the growth, sporangial production and germination in P. splendens was not the same for the growth, sclerotial production and germination in R. lamellifera and it would be expected that the requirements of these fungi in nature might be different.

A temperature range of 25° - 30°C was found to be the most suitable for the growth and sporangial germination in P. splendens and the growth and sclerotial germination in R. lamellifera. The greatest growth in both fungi in the temperature levels investigated was obtained at 30°C (Fig. 6). The fungi did not grow at the higher temperature of 35° or 40°C during the 3 day-duration of the experiment. Similar results were reported by Robertson

(1959a) who found that the greatest mycelial dry weights in P. splendens and R. lamellifera were obtained at 30°C and 29°C respectively. Braun (1925) also found that the best growth and sporangial production in P. splendens were obtained between 27° and 30°C with the optimum at 30°C. Van der plaats-niterink (1969) reported that there was no growth in P. splendens at 5°C or over 35°C and that the fungus required a temperature range of 25-30° for optimal growth. Similar observations had earlier been reported by Waterhouse and Waterston (1966).

Studies on the effects of the compound fertiliser (NPKMg) used in oil palm culture showed that between 1-10,000 p.p.m. it appeared to have no effect on the growth and sporangial germination but suppressed sporangial production in P. splendens in vitro (Table 19). Compound fertiliser was found to be generally inhibitory to the linear growth, sclerotial production and germination in R. lamellifera (Table 24). It is tempting to infer from these results that compound fertiliser should reduce the incidence of the blast disease in the nursery. On the contrary, Robertson (1959a) and Forde et al. (1968) working at the NIFOR Main Station found that compound fertiliser aggravated the blast disease. A similar result was obtained by Chahal et al. (1970) who found that heavy doses of nitrogenous fertilisers (calcium ammonium nitrate or urea) increased the severity of stunted growth of wheat caused by Pythium irregulare Buis. It is postulated that the observed effect of the

fertiliser is probably due to its effect on the host and not on the parasite. The fertiliser probably increased the susceptibility of the host to infection by stimulating growth and the production of a root system composed predominantly of young and tender roots.

All the experiments carried out in this investigation to induce the formation of zoospores in P. splendens were unsuccessful even when the same conditions which induced their formation in P. palmivora (Weststeijn, 1964 and 1966) were applied. The sporangia of P. splendens were found to germinate invariably by the production of germ tubes and up to 7 germ tubes were produced per sporangium. Similar results were obtained by Braun (1925) who reported that P. splendens did not produce zoospores even when the same conditions which induced their formation in P. complectens were applied. Furthermore, he found that the sporangia germinated by germ tubes and observed that a sporangium produced up to 6 germ tubes when inoculated on rich media.

Experiments carried out to induce sexual reproduction in P. splendens by growing the fungus in different types of agar medium, allowing the cultures to age, interpairing strains of the fungus or growing the fungus on a special beta-sitosterol agar medium were also unsuccessful. Braun (1925) reported that oospores of P. splendens were scarce in culture and that they were found

only sporadically in corn-meal agar. In the same publication, he admitted that the conditions necessary for oospore formation in the fungus were not known. Robertson(1959a and 1959b) also reported the failure of an isolate of Pythium (probably P. splendens) from diseased oil palm seedlings to produce oospores in culture. Van der plaats-niterink (1969) observed that in most isolates of P. splendens none or only very few oogonia were found in culture and attributed the rare occurrence of oogonia to heterothallism. In the same publication, he reported that by interpairing the strains, the fungus produced a heterothallic response in seven out of nine strains while the remaining two strains produced oogonia in single cultures.

Neither the hypha nor sporangium of P. splendens was observed to be parasitised by R. lamellifera. This contradicts the result obtained by Robertson (1959b) who reported that the hyphae and sporangia of P. splendens were parasitised in vitro by R. lamellifera. A similar parasitic relationship was reported by Okaisabor (1968) who found that the hyphae, sporangia and chlamydospores of P. palmivora were parasitised by Botryodiplodia theobromae and that the degree of parasitism varied in different types of medium and under different conditions of temperature.

The present investigations also showed that the blast disease was affected by several factors including the date of planting oil palm seedlings into the

nursery, soil temperature, soil moisture, cropping history, planting practice and colour of polythene bags used for planting. Oil palm seedlings planted into the nursery in April at the beginning of the rainy season in Nigeria suffered a lower incidence of the blast disease than seedlings planted in June, July or August (Table 32). Similar results were obtained with the oil palm (Robertson, 1959a; Robertson et al., 1968) alfalfa and red clover (Chi and Hansen, 1962), cotton (Arndt, 1943), lettuce and cauliflower (Shephard and Wood, 1963). Garrett (1960) attributed this phenomenon to the increasing resistance of the host due to maturation of root tissues. Oil palm seedlings planted in the nursery in September were found to suffer less attack by the blast disease than those planted in June, July or August. This may be attributed to disease escape because most of the roots of such seedlings were growing through the topsoil (which was shown in this investigation to contain fewer propagules of P. splendens than the subsoil) at the onset of the blast disease in October. The same explanation seems applicable to the lower incidence of the blast disease observed in polythene bag nurseries (the polythene bags were filled with topsoil) than in nursery beds and also to the lower incidence of the disease in polythene bag filled with topsoil than in those filled with subsoil (Table 38).

The incidence of the blast disease was found to be higher in non-cultivated

forest soil than in cultivated soils despite the apparent greater abundance of Pythium propagules in the latter (Tables 14 and 35). This was attributed to less vigorous growth in forest soil seedlings and hence a greater susceptibility to the blast disease. Unlike the seedlings planted in the cultivated soil, they were neither irrigated in the dry season nor supplied with compound fertiliser. The slightly higher incidence of the blast disease observed in the fallow than non-fallow soils might be a reflection of a higher inoculum density in the former. Although the inoculum density in the two types of soil was not investigated, the observation that both Pythium and Rhizoctonia can competitively colonise organic matter (which was ploughed into the fallow but not the non-fallow soil) appears to lend support to such a hypothesis.

The planting of oil palm seedlings in polythene bags was found to reduce the incidence of the blast disease particularly when the bags were filled with topsoil (Table 40). Turner and Bull (1967) had earlier made a similar observation in Malaysia. This may probably be attributed to a much lower inoculum density. This method of raising oil palm seedlings is being recommended to oil palm planters in West Africa.

The result of the experiment on the effect of colour of polythene bags on the blast disease indicated that the highest mortality was observed among seedlings planted in green polythene bags and the lowest in the black polythene bags (Table 41). One of the over-riding factors which probably varied in the

polythene bags was temperature due to differential absorption of radiant energy by the colours of the polythene bags. Such differences in soil temperature may cause variations in microbial (including blast pathogens) composition, population and activities in the polythene bags and these, in turn, may account for the differences observed in the level of incidence of the blast disease.

The incidence of the oil palm blast disease was found to increase with soil temperature from October to a peak in November when the mean soil temperature in nursery beds was found to be 84.2°F (29°C) which was about the temperature at which the greatest growth in both P. splendens and R. lamellifera was obtained in vitro (Figs. 6, 10 and 14a). The positive correlation found between soil temperature and the blast disease (Fig. 15) is consistent with the findings of other workers. Turner and Bull (1967) reported that mulching with black polythene which increased soil temperature, induced an increase in the blast incidence in an oil palm nursery in Sabah. Watson (1970b) also found that as soil temperature at the planting depth increased from 65°F to 80°F, the degree of Pythium injury to lettuce increased. Similar results were also obtained by Thomas (1970), Littrell and McCarter (1970) and Briesbrock and Hendrix (1970). A probable explanation for this phenomenon was offered by the work of Leach (1947) who observed that

damping-off also incited by Pythium sp., of spinach, sugar beet and watermelon was severest when soil temperature favoured the growth of the pathogen but not the host.

The observation that the incidence of the blast disease was found to be highest in November when the recovery value of Pythium (as determined by the root baiting technique) from soil was at its lowest level (Tables 11 and 29) does not seem to be anomalous in the light of Menzies' (1970) observation that the severity of a disease may not be clearly related to the size of the pathogen population. It is, therefore, postulated that in the oil palm blast disease, the optimum conditions for infection by P. splendens and R. lamellifera may not necessarily be the same as those for vegetative growth and reproduction.

Watering of nursery bed seedlings in the dry season (October-February) was found to aggravate the blast disease (Table 42). Similar results had earlier been obtained at NIFOR Main Station (Anon., 1967a; Anon., 1969). On the contrary, some other experiments also at NIFOR Main Station showed that overhead irrigation particularly in August and September significantly reduced the incidence of the blast disease (Anon., 1960; Anon., 1961 and Anon., 1968a). It is postulated that oil palm seedlings which were supplied with water in August and September grew vigorously and were, therefore, less susceptible to the blast disease. The higher incidence of the blast disease

observed in watered than non-watered nursery beds in this study is also consistent with the report by Beach in 1949 cited by Wheeler (1969) who found that the severity of attack by Pythium ultimum on peas continued to increase with the percentage soil moisture to the saturation point. Kerr (1964), however, reported that soil moisture did not affect disease incidence directly but its importance was in its influence on the amount of sugar exuded from pea seeds. Similar results were obtained by Flentje (1964) and Flentje and Saskena (1964). Ghaffar and Erwin (1969), Das and Western (1959) however found that root diseases were severe only when plants were subjected to water stress conditions. In these cases, it would appear that the fungal pathogens were able to obtain requisite moisture while moisture stress increased the susceptibility of the host. Jooste (1969), on the other hand, observed that the pathogenicity of Macrophomina phaseoli on sunflower was not affected by the amount of soil moisture. The significant correlation found in this investigation between the blast disease and soil temperature (Table 34) but not between the disease and soil moisture (Fig. 14) suggests that soil temperature may be a more important factor than soil moisture in the epidemiology of the blast disease under the growing conditions in southern Nigeria. This hypothesis was substantiated by the result of the shading (reduction in soil temperature) experiment in which the control of the blast disease in ground beds was

significantly better (P = 0.001) in shaded than in watered plots (Table 46). A similar observation was made by Rajagopalan (1972).

Shading of oil palm seedlings in the nursery during the dry season, particularly from October to December was found to be the most effective method of reducing the incidence of the blast disease (Tables 46 and 48). Shading was found to control the disease irrespective of whether the seedlings were planted in nursery beds or polythene bags. Shading also had no deleterious effect on the seedlings. Furthermore, the seedlings established satisfactorily and were not attacked by the blast disease when they were subsequently planted in the field (Table 47). Bachy (1958) working in the Ivory Coast also found that when seedlings were shaded from October to February, the incidence of the blast disease was considerably reduced. Similar results were obtained at NIFOR Main Station (Anon., 1965a; Anon., 1967a; Anon., 1968a; Anon., 1969; Rajagopalan, 1972), Ogba Farm in the Mid-Western State, Abak in the South-Eastern State of Nigeria, (Gunn et al., 1961), Dahomey (Stessels and Malingraux, 1968) and Malaysia (Turner, 1965). Natural shade under forest vegetation was found to be as effective as artificial shade under pruned palm fronds in the control of the blast disease Fig. 21). This should be of considerable interest to oil palm planters in Nigeria and other West African countries because the method is cheap and simple to adopt.

Although the effects of the various factors on the blast disease have as far as possible been considered separately, these factors seldom operate independently in nature. It is their interaction that produces the end result of disease incidence level. Thus a clearer understanding of the oil palm blast disease should take into consideration the effects of these and other factors operating concurrently on the host, pathogens, host-pathogen relationship and the entire agro-ecosystem.

In laboratory screening of selected chemicals, Fernasan was found to be the most active chemical against P. splendens. The LD_{50} values for inhibition of the growth and sporangial germination in the fungus were found to be 7.5 p.p.m. and 3.7 p.p.m. respectively (Fig. 8). Benlate, on the other hand, was found to exhibit the greatest activity against R. lamellifera. The LD_{50} for the inhibition of the growth of the fungus was found to be 0.1 p.p.m. (Fig. 12). The fungicide completely suppressed growth in R. lamellifera at 0.2 p.p.m. (Table 28). Al-Beldawi and Pinckard (1970) found from in vitro studies that benomyl (Benlate) markedly reduced growth in R. solani at 0.62 p.p.m. and that complete inhibition was achieved at a minimal concentration of 2.5 p.p.m. On the basis of the results from these in vitro experiments, Fernasan and Benlate were selected for nursery trials on the control of the oil palm blast disease. Pentachloronitrobenzene (PCNB)

was included in the nursery trials with the aim of determining the relative importance of P. splendens and R. lamellifera in the aetiology of the blast disease since PCNB is known to control diseases incited by Rhizoctonia and to aggravate those caused by Pythium.

The results of the nursery experiments on the chemical control of the blast disease showed that the incidence of the disease was generally low in both chemically treated and untreated plots (Table 50). The low incidence of the disease even in untreated plots was attributed to partial sterilisation of the soil by baking over an open fire prior to planting. However, Fernasan (thiram as active ingredient) was found to be the most effective chemical in the control of the blast disease. The control of the disease achieved with Fernasan was found to be statistically significant at the 1% level in comparison with untreated plots (Table 50). Munnecke and Mickail (1967) also found that damping-off of pea caused by Pythium ultimum was suppressed by thiram. Richardson (1954) attributed the effectiveness of thiram in protecting seedlings against damping-off to the action of antibiotic substances produced by thiram-resistant soil fungi. These results differ from those of Setliff and Hocking (1967) who found that thiram did not offer sustained protection against damping-off of pine caused by Pythium sp. and it is probable that if antibiotics were produced, they were short-lived.

Benlate was also found in this investigation to reduce the incidence of the blast disease in comparison with untreated (control) plots (Table 50). The fungicide has been reported to be active against numerous fungi in the Ascomycetes (Schroeder and Provvidentil, 1968), as well as Rhizoctonia solani, a Basidiomycete, but not against pythiaceous fungi or bacteria (Delp and Klopping, 1968; Edgington et al., 1971). Al-Beldawi and Pinckard (1970) reported that Benlate controlled damping-off of cotton seedlings caused by R. solani in treated soil in the greenhouse. A similar result was obtained by Al-Beldawi in 1969 cited by Al-Beldawi and Pinckard (1970) who found that three fungicides including benomyl (Benlate) were effective against R. solani on cotton seedlings. The merits of this fungicide include its systemic action and effectiveness at low concentrations.

Other results showed that pentachloronitrobenzene (PCNB) did not control the blast disease. On the contrary, the fungicide appeared to aggravate the disease (Table 50). Brown and Montgomery (1948) reported that PCNB and 2, 3, 5, 6 - tetrachloronitrobenzene were widely used as agricultural fungicides especially for the control of diseases incited by R. solani and Botrytis cinerea Pers. Corden in 1969, cited by Katan and Lockwood (1970), found that PCNB was effective against R. solani, Sclerotium rolfsii Sacc. and Streptomyces scabies but not effective in controlling diseases caused by species

of Pythium or Fusarium. Similar results have been obtained by Campbell (1956), Last (1952), Farley in 1968 cited by Ko (1968). In some instances, the incidence and severity of diseases caused by Pythium or Fusarium were even increased in soil treated with PCNB (Bird et al., 1957 and Gibson et al., 1961). Various hypotheses have been postulated to explain the selective toxicity of PCNB to fungal plant pathogens. The phenomenon has been attributed to selective uptake and accumulation of PCNB by Rhizoctonia (Vredeveld in 1965, cited by Nakanishi and Oku, 1969; Ko and Lockwood, 1968), selective uptake and metabolism of PCNB to pentachloronitroaniline (PCA) and pentachlorothioanisole (Nakanishi and Oku, 1969), suppression of antagonists of PCNB-tolerant pathogens (Gibson et al., 1961; Farley and Lockwood, 1969) and to differential alterations both quantitatively and qualitatively of soil microbial populations in the presence of plant residues (Katan and Lockwood, 1970). The mode of action of PCNB was, however, not investigated in this study. The increase in the incidence of the oil palm blast disease in plots treated with PCNB suggests that Pythium is probably more important than Rhizoctonia in the aetiology of the disease. This hypothesis is supported by the fact that Fernasan which acts mainly against Pythium was found in this investigation to control the blast disease more effectively than Benlate which acts against Rhizoctonia but not Pythium.

Extension work seedlings (dura x pisifera) produced at NIFOR were found to be more resistant to the blast disease than grove palm seedlings (predominantly dura variety) (Table 52). Dura x pisifera cross was also found to be more resistant to the blast disease than tenera x tenera, dura x tenera or dura x dura crosses; the order of decreasing resistance being dura x pisifera; tenera x tenera; dura x tenera; dura x dura (Table 53). These results indicate that the gene(s) for resistance is probably carried by the pisifera parent. The observation that resistance to the blast disease is heritable was made by Anon. (1963), Turner and Bull (1967), Blaak (1969) and Obasola (1972). Results obtained from southern Cameroun indicated that when one of the parents in a cross was highly resistant, the oil palm blast disease was reduced to a low level (Blaak, 1969). Blaak (1969) also reported that pure Deli dura palms were more susceptible to the blast disease than the West African dura and that plant materials originating from the drier areas of Nigeria were more susceptible to the disease than those from theRain Forest Zone. Apart from Deli materials, introductions from Jamaica and Angola have also been reported to be highly susceptible to the oil palm blast disease (Anon., 1962a; Anon., 1963; Turner and Bull, 1967).

Turner and Bull (1967) have aptly pointed out that the oil palm blast disease is best controlled by prevention. The results of the experiments on chemical soil treatment showed that Fernasan and Benlate controlled the blast

disease. Neither of these chemicals was, however, found to be as effective as shading of nursery seedlings during the dry season in reducing the incidence of the disease. Work on the selection of blast-resistant varieties is slow because of the slow growth of the oil palm. In the light of these findings and until resistant varieties of the oil palm are produced for extension work, it is recommended that oil palm seedlings be raised in polythene bags filled with topsoil and that such seedlings should be placed under natural or artificial shade from October to December to minimise losses due to the blast disease in Nigeria.

SECTION VII

SUMMARY

A. Survey of the blast disease in south-western Nigeria

1. Surveys of the oil palm blast disease were carried out between 1967 and 1972 in nine oil palm nurseries in the Western State and four nurseries in the Mid-Western State of Nigeria. The disease was found in all the nurseries.

2. The blast disease was also observed among palms other than the wild or cultivated oil palm (Elaeis guineensis Jacq.) at the NIFOR Main Station, near Benin-City. Among such palms, the disease was found to be more severe on Ptychosperma elegans Blume., Areca catechu Linn., Ptychosperma macarthurii H. Wendl., Areca lynn than on Sabal umbraculifera Mart., Phoenix dactylifera Linn., Caryota mitis Lour., Coccothrinax argentea K. Sch. and Copernicia prunifera Mart.

B. Aetiology of the oil palm blast disease

3. Pythium splendens Braun (Accession No. IMI 149554) and Rhizoctonia lamellifera Small (Accession No. IMI 149556) were consistently isolated from infected roots of oil palm. Both fungi were found, in inoculation experiments, to be the causal organisms of the blast disease on oil palm seedlings.

4. *P. splendens* was found to be more pathogenic on oil palm seedlings than *R. lamellifera* under experimental conditions.

C. Ecological studies on *P. splendens*

5. *P. splendens* was isolated from soil by baiting with discs of live oil palm roots. By using the root baiting technique, it was found that the fungus was not uniformly distributed in soil. Thus the infective propagules of the fungus were found to be more abundant at the ploughline depth of 9 inches than at other depths between 1-36 inches. The recovery value of the fungus was also found to be higher in cultivated than non-cultivated soils. Furthermore, the recovery value of the fungus was higher in soil around the roots of diseased than healthy oil palm seedlings.

6. The recovery value of *P. splendens*, as determined by the root baiting technique, was high in July-October, low in November-January and high again in February-April (but not as high as in July-October). These variations in the recovery value of the fungus were found to correspond with variation in soil moisture but not with soil temperature.

7. The cultural practice of ploughing green and farmyard manure into nursery soil was found to enhance the recovery value of *P. splendens* as determined by the root baiting technique.

8. The propagules of P. splendens could not withstand desiccation. Thus the fungus appeared to have completely lost viability (as determined by the root baiting technique) in a sample of naturally-infested soil which was air-dried and stored at 25-27°C for 16 days.

D. Cultural studies on P. splendens and R. lamellifera

9. The effects of types of agar medium, temperature, compound fertilizer and fungicides on the linear growth, sporangial production and germination in P. splendens were investigated. Czapek-dox and cassava-dextrose agar were found to be the most satisfactory media for the linear growth and sporangial production in P. splendens while the highest sporangial germination was obtained in quaker-oats agar.

10. When the fungus was grown in a temperature range of 15°-40°C, the best linear growth and sporangial germination were obtained in the temperature range of 25-30°C.

11. In agar media impregnated with different concentrations of compound fertiliser (NPKMg) in a range of 1-10,000 p.p.m., linear growth in the fungus was slightly decreased between 1-10 p.p.m. Sporangial production was inhibited at all concentrations and sporangial germination was enhanced between 10 and 100 p.p.m.

12. Cultures of P. splendens which were flooded with sterile distilled

water and cooled under a fan, in an air-conditioned room or in a refrigerator failed to produce zoospores.

13. Sexual structures of P. splendens were not observed in nine types of agar culture medium after a storage period of 2 months at 25-27°C, when isolates were paired in mixed cultures or when the fungus was grown in a special betasitosterol agar medium.

14. When the fungus was cultured in the presence of selected fungicides, Fernasan, Terraclor super x and Terrazole at 50 p.p.m. of the commercial products were found to exert the most inhibitory action against the linear growth, sporangial production and germination.

15. The best linear growth and sclerotial germination in R. lamellifera were obtained on potato-dextrose agar and cassava-dextrose agar while the most satisfactory media for sclerotial production were V8-juice agar and corn-meal agar.

16. Linear growth, sclerotial production and germination in the fungus were best in the temperature range of 25-30°C.

17. When the fungus was grown on an agar medium impregnated with a range of concentrations of compound fertilizer (NPKMg) between 1 and 10,000 p.p.m., linear growth, sclerotial production and germination were generally suppressed by the fertilizer.

18. Sclerotial longevity was highest at a low temperature of 20°C and a relative humidity value of 95%.

19. When P. splendens and R. lamellifera were grown together in the same plate of agar culture medium both fungi grew well in the medium but neither the sporangium nor the hypha of the former fungus was observed to be parasitised by the latter.

20. Out of the fungicides screened against R. lamellifera, Benlate was the most inhibitory to linear growth and sclerotial production. Terraclor super x and Pentachloronitrobenzene (PCNB) were also found to be inhibitory to the fungus.

E. Cultural factors affecting the oil palm blast disease

21. The oil palm blast disease occurred only in the dry season from October of every year to March of the following year. The highest incidence of the disease was recorded in November and December.

22. The incidence of the disease varied from year to year but annual losses of extension work seedlings at the NIFOR Main Station during the period of this investigation varied only between 8.6% and 16.8% with an average of 12.9%.

23. The date of planting seedlings into the nursery was found to influence the onset and level of incidence of the blast disease. The

disease occurred earlier among seedlings planted in April or May than among those planted in August or September. The seedlings with the highest incidence of blast were those planted in June, July and August in which twice as many seedlings were affected as in April, May or September plantings.

24. Soil temperature in polythene bag planting was found to be positively correlated ($r = 0.7758$) with the blast disease at the 1% level of significance. Soil temperature and soil moisture in ground bed planting were not significantly correlated with the disease.

25. Oil palm seedlings which were planted in non-cultivated, secondary forest soil were affected by the blast disease in the same way as those planted in a cultivated wet season nursery at the NIFOR Main Station. The disease appeared to be less severe in cultivated soil, non-fallow soil and topsoil than in non-cultivated soil, fallow soil and subsoil respectively.

26. The incidence of the blast disease was found to be lower in polythene bag than in ground bed plantings. In a comparison of the incidence of the disease in black, green and white polythene bags, the highest incidence was recorded in green and the lowest in black polythene bags.

F. Control of the oil palm blast disease

27. Control of the blast disease by cultural practices, application of

chemicals and the testing of varieties of the oil palm for resistance to the disease was investigated. Shading of nursery seedlings during the dry season (particularly from October to December) was found to be the most satisfactory method of reducing the disease incidence. Planting of seedlings in polythene bags also reduced the blast disease incidence. Watering of ground bed seedlings, however, appeared to aggravate the disease. When the nursery seedlings were planted into the field, they established satisfactorily.

28. In an experiment to control the blast disease by soil treatment with fungicides, Fernasan and Benlate reduced while Pentachloronitrobenzene (PCNB) appeared to increase the incidence of the disease. None of the chemical treatments appeared to be as effective as shading of the seedlings in reducing the incidence of the blast disease. The seedlings planted in soil treated with the fungicides did not show any undesirable side effects and they established satisfactorily in the field.

29. Seedlings derived from hybrid tenera seeds (extension work seeds) were found to be more resistant to the blast disease than grove palm seedlings.

30. Out of four crosses of oil palm tested for resistance to the blast disease, seedlings derived from dura x pisifera cross (extension work

seedlings) were found to be the most resistant while those derived from dura x dura cross were the least resistant to the disease.

SECTION VIII

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SECTION IX

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