

Phytophthoras on rubber ~~4~~Hevea brasiliensis~~5~~;
pathogen taxonomy, survival and disease control

by

Nawagamuwage Indranee Saumyaseeli Liyanage

B.Sc. Agric. (Cey.) M.Sc. (Sri Lanka)

A thesis presented in part fulfilment of the requirements for the
Doctor of Philosophy in the Faculty of Science of the
University of London.

Department of Pure and Applied Biology
Imperial College of Science and Technology
Silwood Park
Ascot
Berkshire
England.

December 1986

ABSTRACT

The morphological characters of 86 Phytophthora isolates from rubber throughout the World and 13 isolates from cocoa were examined critically. Five species were distinguished on rubber : P. palmivora, P. meadii, P. botryosa, P. citricola, P. citrophthora and one currently designated as MF₄.

The occurrence of P. citricola (in Sri Lanka) is the first record of this species on rubber and P. citrophthora is reported for the first time from rubber in the Ivory Coast. The 13 isolates from cocoa included two species: P. palmivora and P. katsurae. The occurrence of P. katsurae (from Ivory Coast) is the first record of this species on cocoa.

Tests showed that isolates of Phytophthora species from rubber could also infect cocoa and isolates from cocoa could infect rubber. Oogonia were produced when isolates from cocoa were paired with isolates from rubber. Both normal and abnormal (aborted) oogonia were formed in such pairings.

Most isolates from rubber in Sri Lanka were of P. meadii, A₁ compatibility type. Oogonia were produced in dual cultures with A₂ strains, their formation being favoured at low temperatures (22°C or below) in the dark. P. citricola produced oospores in both the light and dark at 20-30°C. Chlamydospores were produced readily in V₈-CaCo₃ broth and in an extract from petioles of rubber. They germinated in dilute solutions of selected sugars, amino acids and enzymes but less well in soil extract. Only some young oogonia of P. citricola germinated.

Mycelium and sporangia of P. meadii survived only for short periods (3-4 weeks) in the absence of the host but longer (6 weeks) in petioles placed in soil. Thick-walled chlamydospores formed in petioles buried in soil and remained until these decayed but cultures of P. meadii could not

be obtained when these predominated. Their significance in survival thus remains in doubt.

Of six new fungicides oxadixyl appeared most promising both in in-vitro and in-vivo trials for control of panel disease.

Acknowledgements

I wish to record my sincere thanks to Dr. B.E.J. Wheeler, my supervisor, for his unstinted support, guidance and invaluable help throughout the course of this work. His patience, understanding and encouragement are gratefully acknowledged.

I would also like to express my sincere thanks to Dr. O.S. Peries, the former Director of the Rubber Research Institute for helpful discussions during the period at which field experiments were carried out in Sri Lanka and I am thankful to the Rubber Research Board for giving me leave to carry out Post-Graduate studies.

I am grateful to the World Bank and the Food and Agriculture Organization for providing me financial assistance during the first and the third year and to the Overseas Research Student (ORS) award scheme for providing financial assistance to cover part of the tuition fees.

My thanks also go to the staff of the Plant Pathology Department, Silwood Park - Mrs. P. Tyler, Mr. E.E. Green and Mr. A. Broodbank and colleagues for their help and friendship.

Grateful thanks are due to Mr. S.S. Warnapura for his assistance in field experiments, Mr. W. Amaratunga for help in photography.

Dedicated with love to my husband Lyn and daughters

Sonali, Anali and Dineli

TABLE OF CONTENTS

	Page
ABSTRACT	2
ACKNOWLEDGEMENTS	4
GENERAL INTRODUCTION	13
CHAPTER 1: PATHOGEN TAXONOMY OF PHYTOPHTHORAS FROM	
RUBBER AND COCOA	24
1. Literature review	24
1.1 Historical Introduction	24
1.2 Present criteria	27
1.2.1 Characters for main groups	27
1.2.2 Characters at species level	28
1.2.3 Intermediate characters	30
1.2.4 Characters important for one	
or two species	30
1.2.5 Supporting characters	31
1.2.6 Characters needing further	
examination	32
1.3 Pairings of <u>Phytophthora</u> isolates	32
2. Materials and methods	34
2.1 Isolates	34
2.1.1 From rubber	34
2.1.2 From cocoa	34
2.2 Culture media	34
2.3 Colony morphology on lima bean agar..	36
2.4 Growth rate on agar	36
2.5 Sporangial morphology and pedicel	
length	36

	Page
2.6 Sporulation	36
2.7 Zoospore release, number and size	
of zoospores and pore size	38
2.8 Intra-specific and Inter-specific	
pairing of isolates	38
2.9 Inoculation of rubber petioles	
with <u>Phytophthora</u> species	38
2.10 Inoculation of cocoa pods with	
<u>Phytophthora</u> species	39
3. Results	40
3.1 Isolates from rubber	40
3.1.1 Pedicel length	40
3.1.2 Size, shape and L/B ratio	
of sporangia	40
3.1.3 Colony characters	43
3.1.4 Growth rate	51
3.1.5 Sporulation, zoospore	
characteristics and pore	
size of sporangia	51
3.1.6 Chlamydospore production and	
their size	58
3.1.7 Pairing of isolates	58
3.1.8 Homothallic isolate from	
rubber	61
3.1.9 Identification of species	65
3.2 Isolates from cocoa	67
3.2.1 Morphological characters	67

3.2.2	Abnormal oogonia in pairing of <u>P. palmivora</u> from cocoa and <u>P. meadii</u> from rubber		75
3.2.3	Inoculation of rubber petioles with <u>Phytophthora</u> species		75
3.2.4	Inoculation of cocoa pods with <u>Phytophthora</u> species		76
4.	Discussion		84
CHAPTER 2: STUDIES ON <u>PHYTOPHTHORA</u> ISOLATES OF			
	RUBBER FROM SRI LANKA		91
1.	Introduction		91
2.	Literature review		92
2.1	Oospores and the factors influencing their production and germination		92
2.1.1	Production of oospores		92
	(a) Temperature		92
	(b) Light		92
	(c) Stimulation		93
2.1.2	Germination of oospores		94
	(a) Oospore maturity		94
	(b) Temperature		95
	(c) Light		95
	(d) Enzymes		96
2.2	Chlamydospores and the factors influencing their production		

	Page
and germination	96
2.2.1 Production of chlamydo spores	96
(a) Medium	96
(b) Aeration	96
(c) Light	97
(d) Temperature	97
2.2.2 Germination of chlamydo spores	97
(a) C:N ratio	98
(b) Temperature	98
(c) Age	98
3. Material and methods	99
4. Results	99
4.1 Factors influencing production of	
oospores	99
4.1.1 Light/dark	99
4.1.2 Temperature	99
(a) Pairing of <u>P. meadii</u> isolates	99
(b) Production of oospores in	
<u>P. citricola</u> (homothallic	
isolate	99
4.1.3 Stimulation by <u>Trichoderma</u>	
species	101
4.2 Factors influencing production of	
chlamydo spores	101
4.2.1 Temperature/light	101

	Page
4.2.2 pH	105
4.3 Effects of different substrates on germination of oospores	105
4.4 Effects of different substrates on germination of chlamydospores	107
4.5 Effect of different isolates of <u>P. meadii</u> on rubber petioles	108
5. Discussion	113
CHAPTER 3 SURVIVAL OF <u>PHYTOPHTHORA MEADII</u>	116
1. Introduction	116
2. Materials and methods	119
2.1 Survival of mycelium in soil	119
2.2 Survival of sporangia in soil	120
2.3 Survival of chlamydospores in soil...	121
2.4 Detection of the fungus in soil by plating on selective medium and by baiting with cocoa pods.	121
2.5 Survival in infected rubber petioles in soil.	122
2.5.1 Laboratory sets	122
2.5.2 Field tests	123
3. Results	123
3.1 Survival of mycelium in soil	123

	Page
3.2 Survival of sporangia in soil	127
3.3 Survival of chlamydospores in soil... ..	132
3.4 Detection of <u>P. meadii</u> in soils	
from infected rubber plantations	132
3.5 Survival in infected rubber petioles	
in soil	136
3.5.1 Laboratory tests	136
3.5.2 Field tests	143
4. Discussion	146

CHAPTER 4 : THE USE OF FUNGICIDES TO CONTROL PHYTOPHTHORA

DISEASE IN RUBBER	149
1. Introduction	149
2. Literature review	150
3. Materials and methods	152
3.1 Effect of fungicides on mycelial growth	
of <u>Phytophthora</u> species	152
3.2 Screening of fungicides on nursery	
plants	153
3.3 Effect of fungicides on the control	
of <u>Phytophthora</u> panel disease	155

	Page
4. Results	158
4.1 Effect of fungicides on mycelial growth of <u>Phytophthora</u> species	158
4.2 Screening of fungicides on nursery plants	159
4.3 Effect of fungicides on the control of <u>Phytophthora</u> panel disease	159
5. Discussion	168
GENERAL DISCUSSION	169
REFERENCES	173
APPENDICES	189

GENERAL INTRODUCTION

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell.- Arg., belongs to the family Euphorbiaceae and is native to the tropical rain forests of South America. It was introduced to Ceylon (now Sri Lanka) and Singapore in 1876 from the collections made by Henry Wickham in Brazil. These seedlings formed the basis of the natural rubber industry in Asia which provides most of the World's supply of rubber.

Disease caused by various Phytophthora spp., occurs in varying degrees of severity in all countries where rubber is grown and, in some, results in great economic loss. The fungi involved can infect the young shoots, pods, leaf laminae, petioles, stems and tapping panels, the severity of the symptoms varying mainly with the environmental conditions and clonal susceptibility.

In Sri Lanka, where the disease is attributed mainly to Phytophthora meadii McRae, symptoms develop during the South-West monsoon which extends from May to July. These are first seen on the immature, green pods which are most susceptible. Small lesions, indicated initially by 'pin head' black globules of latex, usually at the basal end of the pod enlarge with continuous wet weather into brown water-soaked areas and correspondingly the globules of latex become bigger and more apparent. The pod surface soon gets covered with a mass of white sporangia (Fig. 1), which are washed by rain down onto the leaves, petioles, young shoots and stems where they cause further infections.

On petioles, these are also usually indicated by a globule of latex at the point of the entry. A greyish to black lesion develops along the petiole (Fig. 2) and at this stage leaves absciss with the petiole attached. Before this, leaflets often become reddish brown. On leaves, circular, brownish water-soaked lesions appear on the lamina with fine



Fig. 1. Mature green rubber pods infected by *P. meadii* showing black globules of latex at the basal end of pods and a mass of white sporangia covering the pod surface

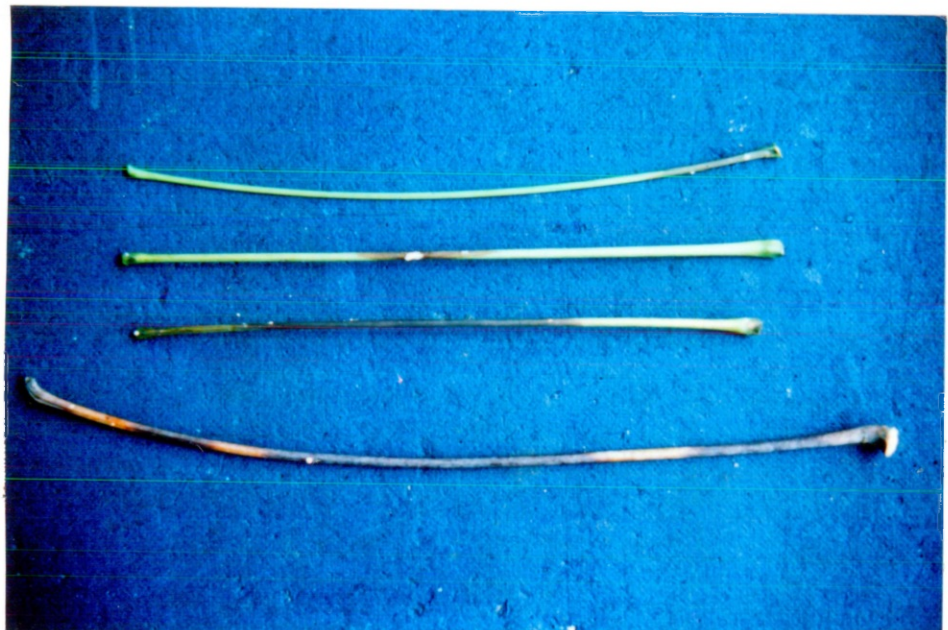


Fig. 2. Rubber petioles infected by *P. meadii* showing greyish-black lesions, with a globule of white latex at the point of the entry.

droplets of coagulated latex arranged in concentric rings. These lesions eventually coalesce to form large irregular areas. Leaflets are easily shed on vigorous shaking.

When shoot apices are infected, the shoots die back. Infections elsewhere on the green shoots result in depressed dark brown areas often with a white matt of sporangia (Fig. 3).

Tapping wounds become infected during the rains when lesions on pods are actively sporulating. A series of sunken, vertically parallel depressions develop just above the tapping cut and sometimes below it as well (Fig. 4). When infected bark is removed, characteristic, distinct vertical black lines are seen on the wood corresponding to the external depressions (Fig. 5). This symptom is commonly called black stripe or black thread. In advanced stages of infection, the bark splits open causing gaping wounds with foul-smelling pads of coagulated latex between the bark and wood. This condition is called canker (Fig. 6). When the disease is neglected or not controlled in the early stages it leads to uneven regeneration of bark resulting in the formation of panel burrs which make subsequent tapping difficult or impossible.

Leaf fall and bark rot of the tapping panel are the two phases of most importance in terms of crop loss, though their relative importance often varies. In India, leaf fall is particularly damaging; yield losses in high yielding clones are reported to be between 38 and 56% (Ramakrishnan, 1961). Incidence of bark rot is negligible as panels are protected by polyethylene rainguards which prevent infection. In Sri Lanka, leaf fall occurs in localized patches with severe sporadic outbreaks (Lloyd, 1963) in wet areas and generally the economic significance is regarded as minimal. However, panel damage is serious enough to warrant cessation of tapping during the monsoon months (Peries, 1964), causing considerable reduction in tapping days.



Fig. 3. Rubber shoots infected by P. meadii showing dark brown lesions.



Fig. 4. Tapping panel of rubber tree infected by P. meadii. Note the vertically parallel depressions above the tapping cut with the exudation of latex.



Fig. 5. Tapping panel of rubber tree infected by *P. meadii* showing vertical, parallel black lines on the wood.



Fig. 6. Extensive damage to the tapping panel of rubber by *P. meadii*, a condition referred to as canker.

In other areas, as in India, leaf fall is most often reported as causing damage. In parts of Northern Malaysia, as much as 80% leaf fall has been recorded on the potentially high yielding clone, RRIM 600 (Wastie, 1973). More recently, mature stands and nursery stock in Brazil have been reported with severe leaf fall (Chee & Kai-Ming, 1985). Leaf fall is also widespread in some regions of Java and Sumatra (Nara et al. (1968), in Thailand (Chee & Greenwood, 1968) and in China, especially Yunan Province where stem canker and black stripe are also reported (Ho et al., 1984).

The cocoa tree (Theobroma cacao L.,) belonging to the family Sterculiaceae, a native of the rain forest of tropical America is cultivated in many countries of the world. It is grown either as a monocrop or as an intercrop especially with rubber, coconut and forest trees.

The importance of the disease caused by Phytophthora spp., in cocoa is well established. Economically, the greatest loss is from pod infection. The extent of damage brought about by infection of the stems, the leaves and the roots is difficult to assess in monetary terms but is probably very considerable. Padwick (1956) considered that the world's mean annual loss from black pod disease amounted to 10%, whereas Opeke and Gorenz (1974) estimated the loss at 20-30% varying according to annual severity. Disease severity varies widely from one country to another. In Malaysia (Chan & Lee, 1973) and Papua New Guinea (Hicks, 1967) losses are negligible or low, while in most of the major producing countries in West Africa and South America the figures are alarming and can amount to 80-90% of the crop (Opeke & Gorenz, 1974).

Phytophthora spp., infect trunk, roots, cushion flowers, flowers, young leaves and pods (Dade, 1927) of the cocoa tree. Cocoa pods are infected in all physiological stages from the cherelle (young pod) stage to mature pod and this disease is commonly referred to as black pod.

The symptoms appear first as small brown spots which enlarge and spread readily to cover the whole pod surface (Fig. 7). A light bloom of whitish sporangia is often seen on the surface. The colour of the infected pod eventually turns dark brown or black in about two weeks and the beans within the pod become unusable. Cankers may arise on the main trunk or in the branches. Externally, symptoms appear as discolouration of bark but is difficult to recognize (Firman, 1974). In advanced stages reddish fluid may exude through cracks in the bark.

Flower cushion infection is closely related with pod infection. Infection may arise from invasion of the subtending cushions by the spread of mycelia from severely infected pods hanging on the trees, and vice versa. Recent investigations have shown that they affect pod setting and survival and damage the cushion itself (Brasier et al., 1981). Blight of leaves, chupons, cuttings, grafts and leaves of cocoa are also common (Manco, 1974). Symptoms are rather similar. They appear first as small blackened spots which develop rapidly to form large necrotic areas. Young shoots and leaves may become blighted and infection of stem and petiole causes dieback.

To date, six species of Phytophthora have been reported as the causal organism of pod rot, leaf fall, shoot dieback and bark rot of the rubber tree. These are P. meadii McRae, P. palmivora (Butl.) Butl., P. botryosa Chee, P. citrophthora (R.E. Sm & E.H. Sm.) Leonian, P. nicotianae var. parasitica and P. phaseoli Thxt.

P. meadii was first described on rubber in India (McRae, 1918) but later work showed that P. palmivora was also present (Thankamma et al., 1968). The presence of P. botryosa in Andaman Islands of India has also been observed (Anon, 1979). P. nicotianae var. parasitica and P. phaseoli Thxt. have also been recorded in India (cited by Ho et al., 1984). Gadd in 1924 reported P. faberi as the causal organism



Fig. 7. Natural black pod infection on cocoa

in Sri Lanka but later work of Waterhouse (1963); Peries & Dantanarayana (1965) corrected its identity as P. meadii. A study of isolates from two rubber growing areas in Sri Lanka showed that both P. meadii and P. palmivora were present (Dantanarayana et al., 1984). P. meadii has also been reported from Malaysia (Chee et al., 1967; Chee & Greenwood, 1968), but later isolates of P. meadii from Malaysia were placed under a new species P. botryosa (Chee, 1969). This species was found to affect rubber trees in Thailand (Tsao et al., 1975). P. palmivora was present in Malaysia (Chee et al., 1967), Indonesia (Nara et al., 1968) China (Ho et al., 1984) and Brazil (Medeiros & Ayres, 1965). Rao et al. (1980) reported the presence of P. capsici on rubber in Bahia, Brazil but later work of Chee and Kai Ming (1985) suggested that there is a strong possibility that the Phytophthora disease in Bahia is caused by P. megakarya.

P. palmivora was formerly thought to be the pathogen attacking cocoa in all areas where cocoa is grown. The taxonomy of the species had been in doubt due to the lack of a detailed description, absence of type cultures and adequate herbarium material of Butler's (1907, 1910) original fungus. The existence of morphological groups within P. palmivora isolates has been shown (Orellana, 1959; Turner, 1960, 1961; Chee 1969; Waterhouse, 1974). Sansome et al., (1975) separated P. palmivora into 'L' and 'S' morphological forms based on the size of chromosomes (Large and Small). At the International Cocoa Phytophthora Workshop, four morphological forms of P. palmivora namely MF₁, MF₂, MF₃ and MF₄ were recognized (Griffin 1977). Brasier and Griffin (1979) found that isolates from cocoa in West Africa conformed to MF₃ and called this a new species, P. megakarya. In this study MF₄ merited special status as a new species; the isolates of which were obtained only from Central and South America. Some workers considered MF₄ to be

identical with P. capsici, but there is no definite proof for this, although MF₄ isolates resemble P. capsici closely in morphology (D.J. Stamps - personal communication). Therefore, in this study isolates that resembled P. capsici will be referred to as MF₄, except when it is cited by other workers.

The information on the distribution of Phytophthora spp., on rubber is limited compared, for example with cocoa where the species are now recorded for most cocoa growing areas. Although several species of Phytophthora are known to affect the rubber tree they have sometimes been incorrectly identified presumably due to lack of standard methods for identification and use of criteria which are variable. No critical study has been done to identify the species of Phytophthora that affect rubber in Indonesia and Ivory Coast. Although the species have been identified from time to time in Malaysia, India, Thailand, China and Sri Lanka, there has been no attempt to examine a wide range of Phytophthora isolates from rubber, worldwide, to compare them under standard conditions and to determine species and morphological variants.

Cocoa is an important intercrop in rubber and succumbs to infections caused by Phytophthora spp.. Petch (1910) suggested that if cocoa and rubber were interplanted, the Phytophthora on one host might infect the other. There is also the possibility of cross compatibility of isolates, a knowledge of which is useful as sexual reproduction would result in the development of new and virulent strains of the fungus. The sexual spores are better adapted to survive under unfavourable conditions.

Specifically, on rubber in Sri Lanka there are gaps in knowledge on some important aspects of survival especially in perennation of inoculum of P. meadii. Availability of this information would allow proper timing of control methods, thereby preventing a build-up of initial inoculum.

This study examined a wide range of Phytophthora isolates from rubber worldwide, and compared them under standard conditions in an attempt to determine species and morphological variants.

The compatibility of different species of Phytophthora of rubber and the compatibility of cocoa and rubber isolates were examined and the pathogenicity of these species on a few recommended rubber clones from Sri Lanka was evaluated.

An attempt was also made to study important aspects of perennation of inoculum, and to evaluate some fungicides for the control of disease on mature and immature rubber plants under field conditions and to screen other candidate fungicides.

CHAPTER 1

PATHOGEN TAXONOMY OF PHYTOPHTHORAS
FROM RUBBER AND COCOA

1. Literature Review

1.1 Historical Introduction

Anton de Bary first coined the name Phytophthora ("plant destroyer") in 1876 (de Bary, 1887), when he described the causal organism of the potato late blight disease which devastated the potato cultivation in Europe. The features that he singled out as characterizing the genus Phytophthora from other fungi known at that time were the branching of the sporangiophore, the shedding of the sporangia, the formation of the zoospores in the sporangium and the germination of the sporangium by zoospores or by a tube. Sex organs (antheridia and oogonia) were not known then. He described the potato late blight fungus P. infestans as the type species for the new genus. For several years P. infestans was the sole representative of this genus. Then more species were described beginning with P. cactorum in 1886, P. phaseoli in 1889 and P. nicotianae in 1896. In these early years there was no critical appraisal of the characters which were of most value in delimiting species but very often the host type was taken into consideration in erecting a species under the genus. For example, P. phaseoli was isolated from bean, P. nicotianae van Breda de Haan from tobacco and P. arecae from areca nut palm. This led Rosenbaum (1917) to embark on a comparative study of species with a view to constructing a diagnostic key.

The main criteria Rosenbaum selected were the nature of the antheridium, whether amphigynous (antheridium encircles the oogonial stalk) or paragynous (antheridium attached on a side at the base of the oogonium), papillum (apical portion of the sporangium) prominent or not, sporangial length-breadth ratio (L/B ratio), presence or absence of chlamydospores and their size and oospore size and their abundance. He also noted that cultures varied with the medium used and the temperature.

The next attempt at a monograph of Phytophthora was that of Tucker (1931). He distinguished species using 11 criteria: ability to grow on certain media, cardinal temperatures usually optimum and maximum, hyphal swellings, abundance of sporangia, papillum prominent or not, presence/absence of chlamydospores and sex organs, type of antheridium, oospore size and pathogenicity determined by inoculation.

In 1934, Leonian produced a key similar to Tucker's after examining 110 isolates. He found that pedicel length (length of hyphae which remains attached to the sporangium when it is shed from the sporangiophore) and growth of isolates in media containing malachite green at different concentrations to be particularly useful for separating species. He concluded that there were not more than three "good" species at that time. Frezzi (1950) also devised a key combining features from both Tucker (1931) and Leonian (1934). Tucker's monograph however, remained the standard work for more than 30 years.

Later, Schwinn (1959) examined 14 criteria in comparative studies. Those that gave some distinction between species were hyphal thickness, hyphal swellings, colony morphology, the antheridium (amphigynous or paragynous), abundance of both sex organs and sporangia on solid media, oospore and chlamydospore size, L/B ratio of the sporangium, growth in relation to temperature and papillae (prominent or not). The pedicel

length was thought to be of secondary importance.

By 1960, the number of criteria used to identify species of Phytophthora had increased and opinions varied as to how many "good" species there were. It was felt that the time had come to produce a 'world' key. The new key (Waterhouse, 1963) was based on studies of cultures received at the Commonwealth Mycological Institute (CMI) over a period of 17 years and of slides left by S.F. Ashby. In this key, the genus was divided into six groups on the following criteria: nature of the sporangial apex (papillum present or not); apical thickening hemispherical, shallow or inconspicuous; the width of the exit pore through which the zoospores are released from the sporangium; abundance of sporangia on solid media; caducity (shedding of sporangia at maturity); internal proliferation of the sporangiophore (sporangia repeatedly produced one inside the other); the nature of the antheridium, whether amphigynous or paragynous and the presence (abundance) or absence of oospores on the host and in culture. The other characters featured in the key for certain species were sporangial dimensions, L/B ratio, branching of the sporangiophore, presence of hyphal swellings, presence or absence of chlamydospores, oogonial size, ornamentation of the oogonial wall, host specificity and cardinal temperatures (optimum and maximum). This revision removed a number of superfluous species that had been described since Tucker's monograph but revived four that he had placed in synonymy namely, P. meadii, P. arecae, P. citricola and P. hibernalis.

A new key by Newhook et al. (1978) attempted to resolve some of the remaining problems. Both the range of variability within a species and the overlapping between species of ranges in individual characters were taken into account. It was emphasised that not all characters listed for a species need be present in a single isolate. Characters were also

grouped into those good for main groups and those useful at species level.

1.2 Present Criteria

1.2.1 For main groups

Some of the characters used by Waterhouse (1963) to segregate species into six main groups (I - VI) were retained by Newhook et al. (1978) as follows:

Apical thickening of the sporangium and exit pore. The sporangium in Phytophthora is always thickened at the apex. This thickening may be hemispheric, shallow or inconspicuous. The width of the exit pore and depth of apical thickening are features that are not subject to variation within a species. The species with hemispheric or shallow thickening generally, have a narrow exit pore and those with inconspicuous thickening have a broader pore.

Caducity of sporangia and pedicel length: sporangia are shed at maturity in some species of Phytophthora which are referred to as caducous species. Three groups of such caducous species can be determined on pedicel length (Zentmyer et al., 1977; Al-Hedaity & Tsao, 1979): (a) short ($5\mu\text{m}$), wide and occluded; (b) intermediate (5-20 μm), thin with protoplasmic contents and (c) long ($>20\mu\text{m}$) thin with protoplasmic contents. These characters seem to be fairly well defined. Species where shedding of the sporangium does not occur (persistent or non-caducous) include all those with non-papillate sporangia (Waterhouse, 1963).

Antheridium amphigynous, paragynous or both: Groups II, IV and VI (Newhook et al., 1978) are characterized by amphigynous antheridia. In groups II all the antheridia are amphigynous, in groups IV and VI the only species exhibiting occasional paragyny are P. hibernalis and P. richardiae. Therefore, amphigyny could be taken as a feature that

varies very little. However, in species with mostly paragynous antheridia there is usually a small proportion (about 1%) of amphigynous antheridia. In groups I and III which have large proportion of both types of antheridia, the numbers are variable.

1.2.2 At species level

Cardinal temperatures: Since Tucker (1931), all keys have used growth at minimum, optimum and maximum temperatures as a useful criterion. High maximum temperature (35°C) is useful for identifying P. nicotianae var. parasitica, P. drechsleri, P. capsici and P. melonis. A low (26°C) maximum temperature distinguishes P. syringae, P. hibernalis, P. infestans and P. lateralis. The production of organs in culture in relation to temperature has proved useful for separation of some species. In P. lateralis, the optimum temperature for chlamydospore production is 24-25°C, which is also approximately the maximum for linear growth. However, the optimum sporangial production is at 14-16°C (Englander & Roth, 1980).

Growth rate: It varies under different conditions but generally slow growth is a useful indicator e.g. P. porri.

Abundance of oogonia in agar media: This character was used by Rosenbaum (1917), Tucker (1931), Leonian (1934) and Frezzi (1950) for P. cactorum and Waterhouse (1963) for P. cactorum and P. hevea.

Sporangial size: Many species share similar or overlapping ranges in sporangial size. However, it is still possible to separate one group with small sporangia (rarely more than 45µm long e.g. P. botryosa, P. katsurae and P. cactorum from another group with large sporangia (often more than 75µm long) e.g. P. palmivora, P. capsici, P. citrophthora and P. cinnamomi.

Sporangial length/breadth ratio: There are species with distinctly large L/B ratio e.g. P. palmivora, P. capsici and some with small L/B

ratio where the sporangia are almost spherical e.g. P. drechsleri and P. arecae.

Oogonial size: The range of oogonial sizes appears fairly consistent among isolates of a species. Restriction to small sizes is especially important for classification of P. botryosa and P. heveae. Large oogonia are characteristic of P. cambivora and P. cinnamomi and the size range extends above 50µm in P. megaspermae var. megasperma but not in P. megasperma var. sojae.

Oogonial wall: In some species the staining of the oogonial wall in young oogonia with aceto-orcein gives differential staining reaction as in P. megakarya and P. palmivora MF₄ but not in P. palmivora MF₁ (Brasier & Medeiros, 1978).

Hyphal swellings in water: The presence of hyphal swellings is a characteristic feature in some species. These are associated with P. syringae, P. porri and P. primulae but not with P. citricola in the same group.

Chlamydospores: Chlamydospores are defined as being completely separated from the mycelium by a basal septum with an inner thickening of the wall. Their presence is useful in separating some individual species such as P. palmivora from P. meadii due to their presence or absence.

Pathogenicity: A record of the source host is obviously an aid to classification. Tucker, (1931) used this in his key. Recently, too, further use has been made of specific pathogenicity for classifying isolates as formae speciales within species such as P. drechsleri f.sp. cajani on Pigeon pea (Kanniyan et al., 1980); P. megasperma f.sp. medicaginis on Alfa-alfa, and P. megasperma f.sp. glycinea on soybean (Kuan & Erwin, 1980).

Culture pattern on agar media: Colony morphology is determined by

growth and branching pattern of the hyphae. Ho (1978) suggested that the branching characteristics at the periphery of a colony might be useful in taxa delineation. Culture patterns however, depend to a certain extent on the media used. The main types of patterns are: radiate (uniformly radiate hyphae), Chrysanthemum (narrow petaloid sectors), Rose or Camellia (broad, rounded petaloid sectors), Uniform (without any obvious pattern) and Stellate (intermediate between radiate and Chrysanthemum).

1.2.3 Intermediate characters

Some characters are present in some species but have particular importance in only a few.

sporangial base tapered: Within a species, sporangia with tapered base may be seen sometimes but in some species the occurrence of this feature is outstanding e.g. P. palmivora MF₄ group which also have the broadest diameter closer to the apex than to the basal septum.

More than one apex: Several species produce sporangia with more than one apex but this character is of outstanding importance only for P. citrophthora and P. citricola.

Oospores markedly aplerotic: In most species the oospore nearly fills the oogonium (plerotic) as in P. katsurae and in some species oospores can be aplerotic (loosely filled) as in P. heveae.

Oogonia with tapered base: This character is especially notable in degree and frequency in P. heveae and P. megakarya, where it contributes markedly to ease of identification. It occurs only in species with amphigynous antheridia.

1.2.4 Important for one or two species

sporangial shape often distorted: Ignoring slight asymmetry, some degree of distortion occurs in 14 species (Newhook et al., 1978). In some, this character is sufficiently common to give it special

diagnostic value e.g. P. citrophthora.

Sporangial collapse after dehiscence: Rands (1922), original description of P. cinnamomi includes sporangial collapse, but the character seems to be encountered in only a few isolates.

Oogonial wall ornamented: This character is particularly important for P. cambivora and P. katsurae, which produce hollow protruberances on the oogonial wall. These are solid and wart-like in P. verrucosa. A single oogonium may have from one to many protruberances of varying sizes.

Oogonial stalk eccentric: This character is uncommon and is a feature that contributes to the maintainence of P. nicotianae var. nicotianae as a separate taxon.

Chlamydospores lateral, sessile: This character is prominent and unique for P. lateralis and its presence makes identification easy and positive.

Hyphal swellings on agar: Coralloid hyphae on agar, especially noticeable at the margin of the colony, are diagnostic for P. cinnamomi. They could be spherical, botryose and large.

Sporangia in clumps: This character is unique to P. botryosa. Chee (1969) recorded clumps of sporangia on culture, visible to the naked eye, with up to 970 sporangia per square millimeter.

Oogonia clustered: The abundant oogonia formed in culture by P. heveae often occur in close groups.

Absence of antheridia: A new species recently described as P. insolita (Ann & Ko 1980) produced oogonia and oospores without antheridia. It is the only known species to do so.

1.2.5 Supporting characters

These characters cannot by themselves be used as primary diagnostic features, but they assist to confirm the identification of certain species.

Hyphal diameter: There can be specific differences in the fineness and coarseness of hyphae. P. cinnomomi and P. capsici have comparatively wide hyphae (7-8µm) whereas in P. palmivora MF₁ hyphal width rarely exceeds 5µm. Another distinguishing feature sometimes is the presence of swellings at the point of branching in a hyphae as in P. meadii, and P. citrophthora.

Antheridial Size: The antheridia of many species are similar in size and shape, but ^{those of} some species are characteristically small (P. heveae) or large (P. cambivora and P. fragariae).

1.2.6 Characters needing further examination.

There are characters such as intercalary sporangia, two-celled elongated antheridia, oospore germination, oogonial wall ultrastructure, chlamydospore wall thickness, amount of aerial mycelium, stimulation of the formation of sex organs by Trichoderma spp., and several other finer characteristics that could be looked into in detail to assist identification and classification (Waterhouse et al., 1983).

More recently, Gallegly (1983) has proposed an entirely new system of classification beginning with the characteristics of sexuality rather than with morphological characters. He suggests that the three major groups of homothallic paragynous, homothallic amphigynous and heterothallic amphigynous species so far encountered should be subdivided by genetic relationships, sexual and asexual morphology, and host-specific pathogenicity. Differences in chromosomal characters, serological relationships and protein patterns would also be important in re-defining species.

1.3 Pairings of Phytophthora isolates.

Some species of Phytophthora exist as 2 mating types (heterothallic). Heterothallic species form oospores when paired with isolates of the opposite mating type belonging to the same species or

when paired with compatible isolates of other species. Ashby (1922) was the first to find that isolates of P. palmivora that did not produce oospores in single culture could do so when paired with a compatible strain of the opposite mating type. This behaviour was subsequently found in a number of other species. In P. infestans the existence of two mating types was demonstrated by Smoot et al. (1958) and Galindo and Gallegly (1960).

Most self-sterile isolates of the heterothallic species, (those ^{only} forming oospores when paired with isolates of the opposite mating type belonging to the same species, also forms oospores when paired with compatible isolates of other species. As early as 1911 Clinton observed oospores when P. infestans was paired with P. phaseoli and claimed that it was the result of hybridization. More recently, an isolate of P. drechsleri of A₂ mating type from potato was found to induce oogonia in P. infestans (Skidmore et al., 1984). Kaosiri et al. (1980) showed that the viability of oospores produced in interspecific pairing is low. Although hybridization may occur frequently within and between species, abortion of hybrid oospores could occur before or during germination (Sansome et al., 1979; Savage et al., 1968).

2. Materials and Methods

2.1 Isolates

2.1.1 From rubber

Thirty-four isolates were obtained from infected rubber in Sri Lanka in July 1983 and a further nine isolates were obtained from the culture collection of the Rubber Research Institute of Sri Lanka (RRISL). These were collected in 1961-1972. In response to requests a total of 86 isolates were received from Brazil, China, India, Indonesia, Ivory Coast, Malaysia and Thailand. Details of the cultures are given in Table 1.

2.1.2 From cocoa

Ten isolates (Nos. 404, 447, T1, M4, M7, K3, K11, Y3, Y14, Y20) were obtained in July 1983 from cultivated cocoa in the central region of Sri Lanka. Two isolates (DIN 4 and PC) were sent from Ivory Coast and one isolate (26B) was sent from Brazil. All isolates were from infected cocoa pods.

2.2 Culture media

Various media were used for particular purposes:

- (a) Lima-bean agar (LBA): assessment of growth rate, sporulation and sporangial morphology.
- (b) V8 juice agar (V_8) : routine subculturing, testing compatibility of isolates.
- (c) V8 juice $CaCo_3$ broth (clarified V_8 juice) and Petiole extract with $CaCo_3$: production of chlamydospores.
- (d) $P_{10}VP$ medium: Selective isolation of Phytophthora spp., from soil and plant parts in studies of pathogen survival.

Details of media preparation given in Appendix 1.

Table 1. Details of Phytophthora isolates from rubber

Country	Source	Substrate	Isolate no.
Sri Lanka	Woodend Estate	Petiole	1P,2P,4P,5P,444
		Leaf	4L,5L
	Elston Estate	Petiole	6P,21P,24P
		Leaf	3L,21L,26L
		Shoot	26S
		Petiole	7P,25P
	Mahaoya Estate	Leaf	25L
		Petiole	11P
	Nakiyadeniya Estate	Leaf	11L
		Bark	12B
		Petiole	13B
	Stokesland Estate	Bark	14P
	Homadola Estate	Petiole	16P,17P
	Mapalagama Estate	Leaf	16L
		Petiole	18P
	Bentota Estate	Leaf	18L
		Petiole	20P,22P
	Frocester Estate	Leaf	22L
		Petiole	23P
	Moralioya Estate	Leaf	27L
		Petiole	28P
	Peenkanda Estate	Bark	29B
	Wagga Estate	Petiole	361,362,365
	Dartonfield Estate	Shoot	126
	Talgaswela Estate	Petiole	9P
	Yahalakele Estate	Petiole	434
	Glassel Estate	Shoot	444
	Ruwanwella Estate	Petiole	445
	Neuchattle Estate	Petiole	462
Brazil	Bahia	Petiole	1,2,51B,23B
	Una	Petiole	3
		Shoot	4
		Shoot	NS1,NS2,NS4,NS5
China	Unknown	Unknown	8220,8221
India	Kottayam	Petiole	81,100,103,105,109
Ivory Coast	Unknown	Petiole	152H
		Leaf	151C
		Seedling	271C
		Pod	PP27
		Bark	531C
Indonesia	Medan (North Sumatra)	Unknown	PPI
	Bulong (Central Java)	Unknown	PPII
Malaysia	Unknown	Petiole	1075,1083,1117,1200,1223,1303,1334,1337,1339,1350,1438,1439,1440
		Bark	1015,1093,1145,1321
		Soil	1071,1072.

2.3 Colony morphology on LBA

Isolates were grown on plates of LBA (15 ml in each) for 6 days at 25°C in the dark. Cultures were viewed against a black background and colony morphology was assessed in terms of aerial mycelium and presence/absence of petaloid characters, as follows:

Aerial mycelium: - None; + sparse; +/++ sparse, but some in the region of the inoculum; ++ moderate; ++/+++ moderate to dense, patchy; +++ dense.

Petaloid nature: Uniform (absent), faintly stellate, stellate, distinctly stellate.

2.4 Growth rate on agar

Plates with 15 ml LBA were each inoculated centrally with a plug (6mm diameter) taken from the edge of a 4-day old culture on LBA. These were incubated at 28°C with a 12h/12/h light-dark regime. At 24h intervals over 6 days two measurements of colony diameter were made at right angles to each other. There were three replicate plates for each isolate.

2.5 Sporangial morphology and pedicel length.

Sporangia were obtained from the plates used to measure colony growth. After 7 days, six plugs (each 6mm diameter) were removed at the positions indicated in Fig. 8, which are either 15 or 25mm from the original inoculum. These plugs were shaken in a Universal Container with 3ml sterile water and a few drops of lactophenol/cotton blue added. The length, breadth and pedicel length of 50 sporangia were measured using an eye piece graticule calibrated with a stage micrometer.

2.6 Sporulation

Colonies of each isolate were grown for 7 days on two LBA plates at 25°C with 12h light/12h dark per day as in 2.4 above. Nine, 6mm plugs were then removed from each plate at the positions shown in Fig. 9.

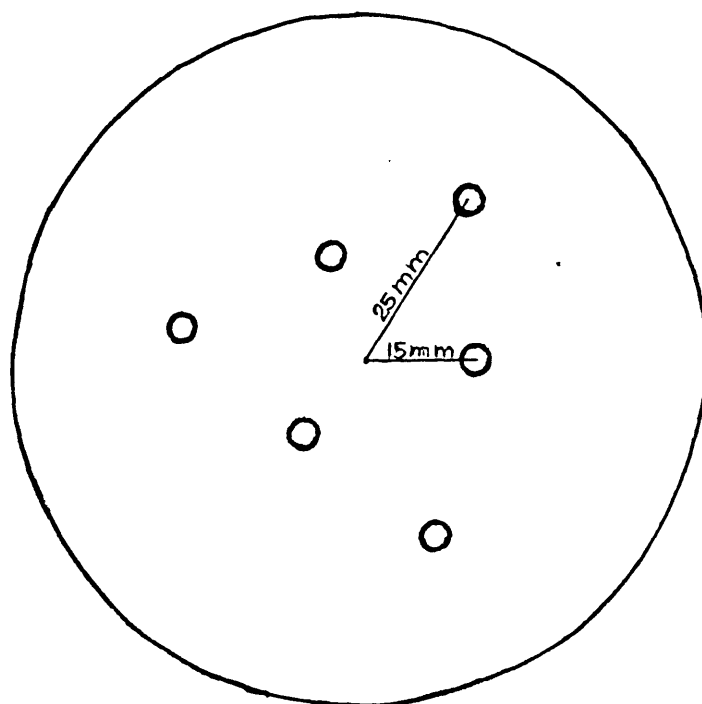


Fig. 8. Positions on the agar plate from where the inoculum plugs were removed for morphological studies of sporangia.

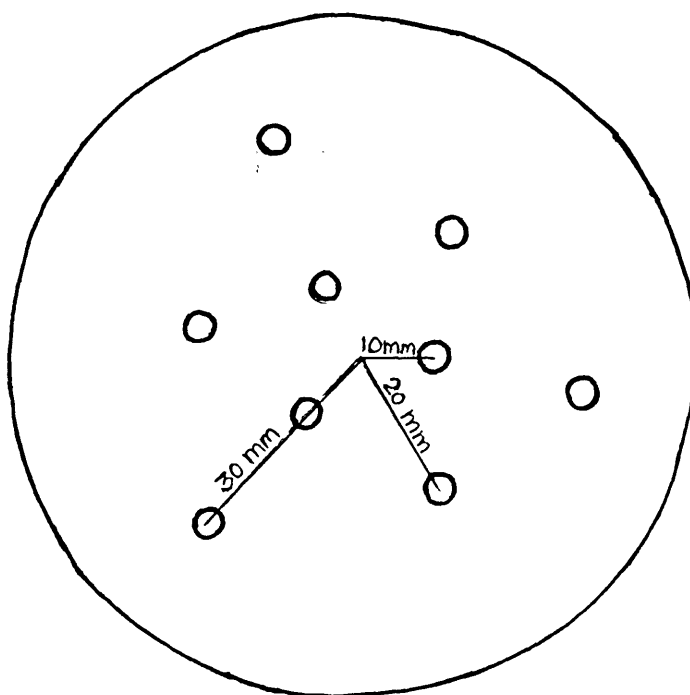


Fig. 9. Positions on the agar plate from where the inoculum plugs were removed for sporulation studies.

Each plug was placed in a drop of lactophenol/cotton blue on a slide which was heated gently to melt the agar. Then by placing a coverslip and applying gentle pressure, a preparation was obtained suitable for counting sporangia in three random microscopic fields. sporangia of each isolate was thus assessed in a total of 54 microscopic fields.

2.7 Zoospore release, number and size of zoospores and pore size.

Suspensions of sporangia were obtained from the plates in 2.6 above by placing five, 6mm plugs in 5ml sterile distilled water in a Universal container and shaking these vigorously. The containers were kept at 20°C for 15 min to induce zoospore formation in sporangia, then incubated at 25°C for 5-10 min to release zoospores and drops of the suspension were examined. The number of zoospores released per sporangium was recorded for 20 sporangia and the diameter of 50 zoospores and the pore size in 10 empty sporangia were measured.

2.8 Intra-specific and inter-specific pairing of isolates.

Isolates were paired in all possible combinations on plates, each containing 15 ml V₈ agar. These were first incubated for approximately 48h at 25°C in continuous light to encourage rapid growth. When they had just begun to meet, the plates were left at 20°C in the dark for 2 weeks. Then five 6mm plugs were removed from each plate along the line where the two isolates met. The plugs were mounted in lactophenol cotton blue on slides. The number of oogonia in 50 microscopic fields were recorded for oogonial frequencies and oogonia size was determined by measuring the diameter of 25 oogonia.

2.9 Inoculation of rubber petioles with Phytophthora species

Tender petioles approximately 12-14 day-old were removed from plants grown in the green house. These were surface sterilized with 2.5% sodium hypochlorite and washed in three changes of sterile distilled water. Ends of petioles were dipped in wax to prevent 'end

rot'. These were placed in germinating trays lined with moist tissue paper. Each petiole was inoculated centrally, with a strip of sterilized absorbent cotton wool 1 cm long and 4 mm wide soaked with 10 drops (0.1 ml) of inoculum from a standardized zoospore suspension of concentration 10^4 zoospores/ml. Length of lesion was measured, 4 days after incubation at 25°C.

2.10 Inoculation of cocoa pods with Phytophthora species.

Mature cocoa pods (variety Amelonado) were surface-sterilized with 2.5% sodium hypochlorite and washed in several changes of sterile distilled water. Three plugs each of 10 mm diameter were removed from 3 positions equidistant from each other, at the centre of the pod. Ten mm diameter inoculum plug from the edge of a 4-day old culture was placed at positions on the pod where the plugs were removed. The inoculated area was sealed with sellotape and the pods were incubated in germinating trays at approximately 25°C. A single pod was used for each species. Horizontal and vertical dimensions of the lesions were measured after 5 days.

3. Results

3.1 Isolates from rubber

With the exception of a single isolate (Isolate no. P0), all isolates had sporangia which were markedly papillate and they only produced amphigynous oogonia in dual cultures. Thus, they could be assigned to Group II in the key of Newhook et al. (1978).

3.1.1 Pedicel length

Of the morphological features examined, pedicel length most clearly divided the isolates preliminarily into groups A,B,C,D and E (Table 2). Group A isolates had distinctly short pedicels (Fig. 10a) with mean pedicel length ranging from 2.5 μm - 3.4 μm and most individual isolates varied between 0.75 μm - 7.5 μm .

Although some isolates were assigned to groups B and C on their pedicel length, the difference between the isolate with the highest pedicel length in Group B (Isolate no. HYN2T) and the isolate with the shortest pedicel length in group C (Isolate no. 105) was not marked. Group D isolates had distinctly long pedicels (Fig. 10b), the mean pedicel length varied between 28.4 μm - 40.9 μm . This clearly distinguished Group D isolates. Some of the isolates produced sporangia which dehisced without a definite pedicel length. In these a large proportion were without a pedicel and some pedicels varied from 2.5 μm to 40 μm . These isolates had sporangia of variable shapes and many were bi-papillate (Fig. 10c) unlike the sporangia in isolates of groups A, B, C and D. Based on the above characters, these isolates were assigned to a separate group E.

3.1.2 Size, shape and L/B ratio of sporangia.

To strengthen the above groupings based on the pedicel length, further characters of individual isolates were examined. One such character was the L/B ratio of the sporangium, but this was very variable (Table 3). The L/B ratio therefore, could not be considered to

Table 2: Pedicel length of Phytophthora isolates from rubber

Pedicel length (μm)			Pedicel length (μm)		
Mean ^a	Range	Isolate no.	Mean ^a	Range	Isolate no.
Group A					
2.5	1.25-7.5	1440	2.8	0.75-7.5	1083
2.6	1.25-7.5	1438	2.8	1.25-7.5	1093
2.6	1.25-5.0	1015	2.8	0.75-7.5	PPII
2.6	0.75-5.0	1072	3.0	2.5-5.0	1145
2.6	0.75-5.0	1200	3.1	1.25-5.0	1321
2.6	2.5-5.0	1339	3.1	1.25-5.0	1350
2.6	0.75-5.0	8221	3.2	1.25-5.0	1439
2.8	0.75-5.0	1337	3.4	1.25-5.0	1071
Group B					
6.4	1.25-12.5	1223	9.3	2.5-13.8	TRT1
7.4	5.0-10.0	1075	9.3	3.8-15.0	1303
7.4	5.0-12.5	1117	9.4	2.5-15.0	HYN2T
7.9	5.0-13.8	HY03			
Group C					
10.5	5.0-22.5	105	12.1	7.5-30.0	18P
11.0	5.0-25.0	1334	12.3	5.0-33.8	361
11.1	5.0-25.0	462	12.4	5.0-30.0	26S
11.1	5.0-25.0	434	12.4	5.0-20.0	4L
11.2	5.0-25.0	14P	12.4	5.0-30.0	24P
11.3	5.0-25.0	13B	12.6	5.0-25.0	22L
11.3	5.0-35.0	362	12.8	5.0-25.0	5L
11.3	5.0-22.5	17P	12.9	5.0-25.0	365
11.5	5.0-20.0	16P	13.0	5.0-22.5	23P
11.5	5.0-25.0	25L	13.2	5.0-25.0	6P
11.9	5.0-20.0	16L	13.2	5.0-25.0	445
12.0	5.0-20.0	12B	13.3	5.0-30.0	1P
13.3	7.5-25.0	456	14.3	5.0-25.0	11L
13.3	5.0-30.0	444	14.4	7.5-20.0	11P
13.4	5.0-30.0	9P	14.5	5.0-30.0	27L
13.5	5.0-25.0	22P	14.6	5.0-35.0	441
13.6	7.5-22.5	4P	14.7	3.0-30.0	3L
13.6	7.5-25.0	20P	14.8	5.0-25.0	18L
13.6	5.0-30.0	29B	14.9	5.0-30.0	28P
13.6	7.5-22.5	126	15.8	7.5-30.0	7P
13.7	5.0-33.7	25P	16.0	7.5-30.0	21L
14.1	7.5-35.0	2P	16.3	5.0-25.0	100
14.1	5.0-30.0	26L	16.5	7.5-28.8	81
14.1	7.5-30.0	21P	18.2	5.0-28.8	103
14.2	5.0-39.5	5P	18.4	10.0-40.0	109

Table 2 (Contd.)

Pedicel length (μm)			Pedicel length (μm)		
Mean ^a	Range	Isolate no.	Mean ^a	Range	Isolate no.
Group D					
28.4	17.5-60.0	51B	29.7	12.5-97.5	NS4
28.5	12.5-60.0	1	29.9	12.5-97.5	NS5
28.6	12.5-55.0	NS1	32.0	12.5-110.0	2
28.9	12.5-72.5	NS2	32.6	12.5-55.0	4
29.7	17.5-97.5	3	40.9	17.5-65.0	23B
Group E					
14.5	0 - 17.5	152H	12.3	0 - 35.0	27IC
12.6	0 - 35.0	PP27	14.8	0 - 27.5	53IC
14.0	0 - 37.5	15IC	16.3	0 - 40.0	8220
			14.6	0 - 37.5	PPI

^a Each value is a mean of 50 sporangia.

be of much value in separating the isolates into groups. For example, some L/B ratio values in Group A (isolate no. 1321, 1200 and 1350) were higher than the L/B ratio for Group B isolates. Also, the range of values from 1.41 - 2.31 in Group A, 1.47 - 2.47 in Group B, 1.29 - 1.78 in Group C and 1.53 - 1.76 in Group E suggested greater variability in shape. The sporangia in Group B isolates were distinctly smaller in size compared to those of other isolates (Fig. 10d). Although small in size, the length of the sporangium sometimes overlapped with the lengths of the sporangia in Group C isolates. However, the breadth of the sporangium distinguished the Group B isolates from those of Group C and all other groups. The mean breadth of the sporangium for Group B isolates varied from 11.28 μm - 18.0 μm and in all other isolates it was higher than 23.6 μm (Table 4). The isolate no. 1334 which was placed in Group, C on the pedicel length character was therefore, placed in Group B, on the basis of the mean breadth value of the sporangium (15.59 μm).

3.1.3 Colony characteristics.

Some of the morphological features such as the petaloid nature of the isolates and the presence or absence of aerial mycelium helped in the grouping of some isolates whilst in others these characters were variable and did not provide further evidence of the groupings already established on the pedicel length. Some isolates in groups A, B and C had no petaloid character, some were faintly stellate, stellate or distinctly stellate (Fig. 11a & b).

All isolates in Group D except one were stellate while group E isolates were without any stellate pattern, (Table 5).

Isolates of Group B, D and E generally had moderate to abundant aerial mycelium (Fig. 11c, d, e) whereas in other groups varying amounts of aerial mycelia were present (Table 6). It was also noted that the 4 isolates in Group C received from India had moderate to dense aerial

Table 3. Length/breadth (L/B) ratios of sporangia of Phytophthora isolates from rubber

L/B ratio			L/B ratio		
Isolate			Isolate		
Mean ^a	Range	no.	Mean ^a	Range	no.
Group A					
1.41	1.25-2.0	1339	1.48	1.5 -2.4	PPII
1.43	1.36-2.14	1438	1.61	1.5 -2.33	1015
1.44	1.25-2.18	1145	1.65	1.5 -2.74	1071
1.44	1.25-2.33	1093	1.80	1.67-2.4	1337
1.44	1.25-2.5	1072	1.81	1.5 -2.12	1439
1.47	1.3 -2.33	1440	2.00	1.73-3.0	1321
1.47	1.3 -2.25	1083	2.02	1.67-2.57	1200
1.48	1.3 -2.25	8221	2.31	2.0 -3.57	1350
Group B					
1.47	1.25-2.87	1075	2.13	1.50-2.87	1303
2.03	1.55-2.40	1117	2.13	1.50-2.87	HYN2T
2.06	1.50-2.75	HYO3	2.47	1.83-3.17	1223
2.13	1.50-2.79	TRT1			
Group C					
1.29	1.14-2.0	18L	1.52	1.14-2.0	126
1.43	1.30-1.75	365	1.53	1.25-2.0	105
1.43	1.30-1.75	22P	1.53	1.14-1.75	26L
1.44	1.11-1.75	24P	1.54	1.14-1.75	20P
1.45	1.14-2.00	21P	1.55	1.14-2.0	13B
1.46	1.15-1.89	5P	1.55	1.15-2.12	18P
1.51	1.05-2.0	362	1.55	1.31-2.12	3L
1.51	1.30-2.10	462	1.55	1.05-2.1	16L
1.51	1.23-1.92	109	1.56	1.05-2.0	25L
1.52	1.29-1.73	17P	1.56	1.2 -2.0	103
1.57	1.05-2.0	14P	1.64	1.21-2.15	2P
1.57	1.25-2.1	23P	1.65	1.42-2.15	26S
1.58	1.14-2.0	22L	1.65	1.3 -2.0	6P
1.58	1.33-1.85	441	1.65	1.38-2.15	11P
1.59	1.17-2.1	4L	1.67	1.44-2.17	7P
1.59	1.11-1.73	445	1.69	1.25-2.09	9P
1.59	1.29-1.75	100	1.70	1.21-2.33	1334
1.60	1.17-2.08	11L	1.71	1.25-2.15	4P
1.61	1.33-2.08	12B	1.71	1.29-2.1	29B
1.61	1.22-2.1	81	1.72	1.3 -2.0	28P
1.61	1.04-2.12	361	1.72	1.8 -2.15	21L
1.61	1.33-2.06	27L	1.74	1.04-2.55	25P
1.62	1.14-2.0	5L	1.75	1.05-1.73	434
1.62	1.31-2.1	444	1.78	1.17-2.09	1P
1.64	1.42-2.08	16P			
Group D					
1.77	1.36-2.14	23B	1.89	1.56-2.25	1
1.82	1.56-2.12	NS5	1.89	1.24-3.57	2
1.86	1.36-2.57	NS4	1.92	1.65-2.67	3

Table 3 (Contd.)

L/B ratio		Isolate	L/B ratio		Isolate
Mean ^a	Range	no.	Mean ^a	Range	no.
Group D (Contd.)					
1.87	1.18-2.33	4	1.94	1.13-3.0	NS1
1.88	1.67-2.12	NS2	2.02	1.53-3.0	51B
Group E					
1.53	1.28-2.3	53IC	1.69	1.25-2.25	27IC
1.54	1.2 -2.25	1527	1.7	1.17-2.0	15IC
1.55	1.3 -2.0	PP27	1.76	1.55-2.0	8220
1.57	1.36-2.5	PPII			

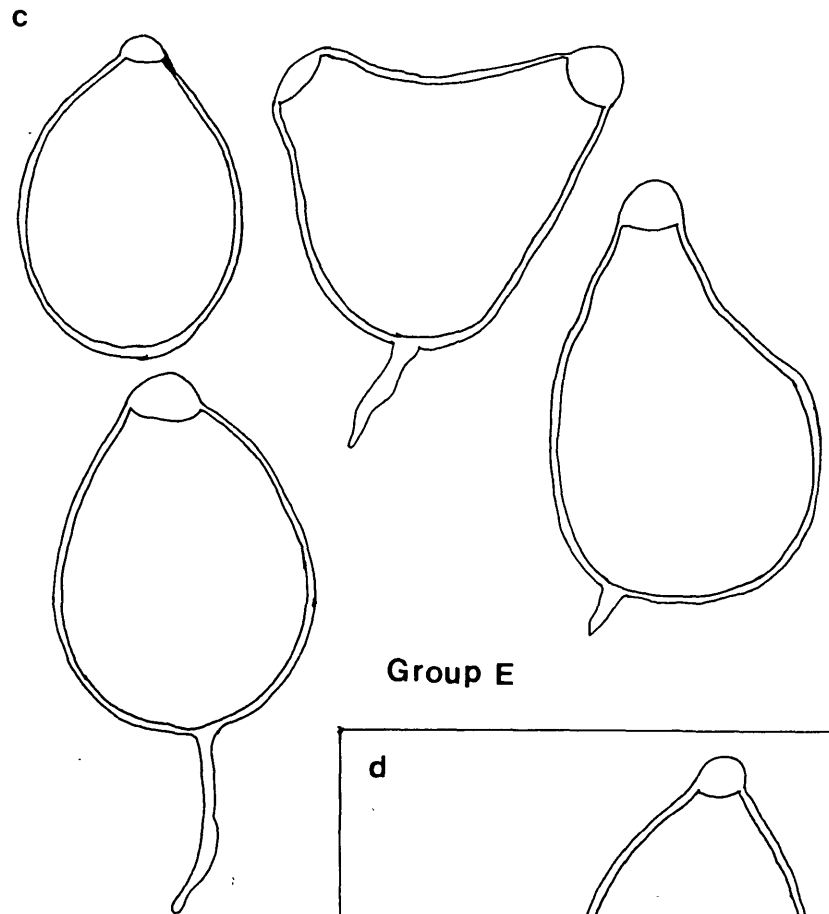
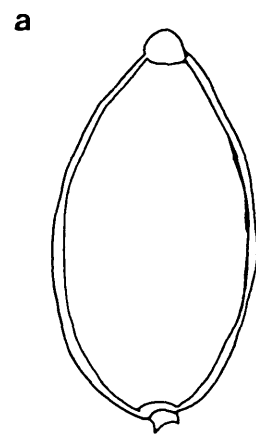
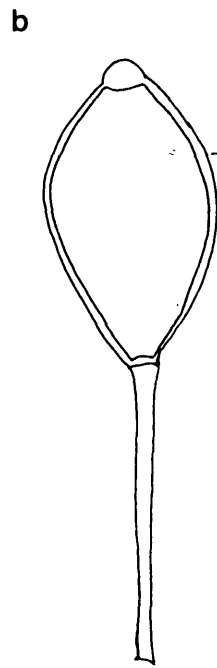
^a Each value is a mean of 50 sporangia.

Table 4. Sporangial breadth of selected Phytophthora isolates from rubber

	Mean sporangial breadth ^a (μ m)	Isolate no.
Group A	24.81 $\pm$ 0.69	1071
	32.71 $\pm$ 0.63	1440
Group B	11.28 $\pm$ 0.32	1303
	11.51 $\pm$ 0.34	HYN2T
	11.92 $\pm$ 0.38	TRT1
	13.07 $\pm$ 0.17	1117
	13.70 $\pm$ 0.28	1223
	15.94 $\pm$ 0.23	HY03
	18.00 $\pm$ 0.44	1075
Group C	36.3 $\pm$ 0.62	105
	15.59 $\pm$ 0.38	1334
	33.20 $\pm$ 0.66	109
Group D	23.6 $\pm$ 0.63	51B
	25.5 $\pm$ 0.67	23B
Group E	31.47 $\pm$ 0.69	152H
	36.52 $\pm$ 0.72	PP27

^a Each value is a mean of 50 sporangia.

Fig. 10 a,b,c & d Sporangial shapes, size and pedicel lengths
of isolates in groups A,B,C and D.



25 μ m

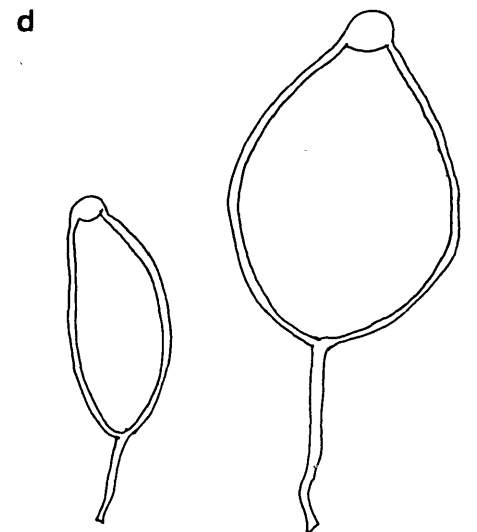


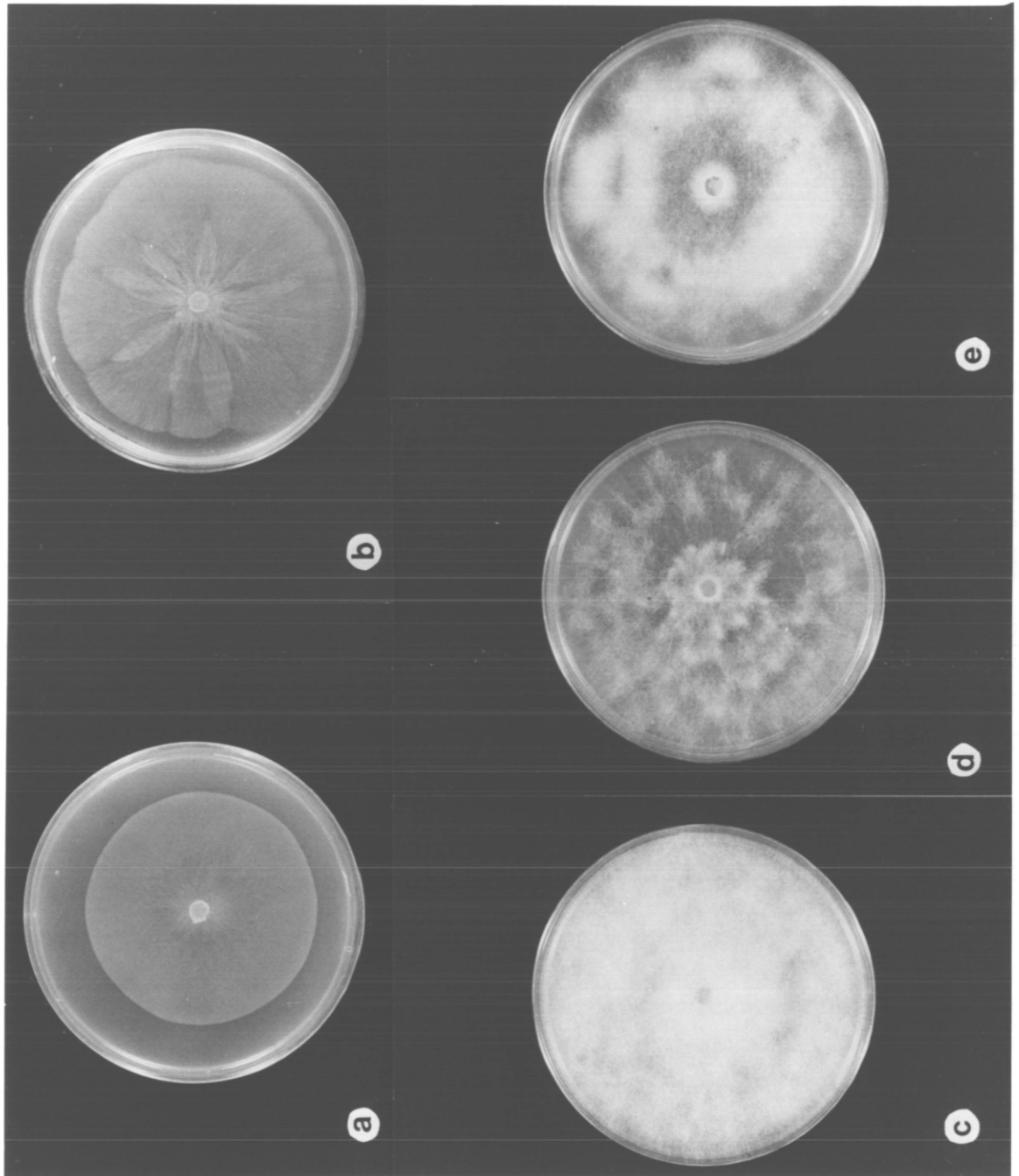
Table 5. Petaloid characters of Phytophthora isolates from rubber.

	Isolate no.	Petaloid character ^a
Group A	1339, 1438, 1093, 1072, 1440, 1083)	
	1071, 1337, 1439, 1200, 1350)	Uniform
	1145, 1015)	Faintly stellate
	1321)	Stellate
Group B	1075, 1117, 1303, 1223, 1334)	Uniform
	HY03, TRT1, HYN2T)	Faintly stellate
Group C	365, 21P, 5P, 362, 109, 126)	
	105, 13B, 3L, 103, 23P, 445)	Uniform
	100, 81, 361, 5L, 444, 6P)	
	7P, 4P, 1P)	
	4L, 22P, 24P, 17P, 22L, 11L)	
	11P, 21L, 25P)	Faintly stellate
	18L, 25L, 14P, 27L, 2P, 29B)	Stellate
Group D	28P)	
	462, 26L, 20P, 18P, 16L, 16P)	Distinctly
	12B, 26S, 9P, 434)	stellate
Group E	51B)	Uniform
	1, 2, 3, 4, NS1, NS2, NS4, NS5)	Stellate
Group E	PP27, 152H, 15IC, 27IC, 53IC)	Uniform
	8220, PPI)	

^a Based on growth on LBA after 6 days at 25°C in the dark. Assessed as uniform (absent), faintly stellate, stellate, distinctly stellate.

Fig. 11 a,b,c,d, & e. Cultural characteristics of isolates

- (a) Stellate pattern absent (uniform)
- (b) Distinctly stellate
- (c) Dense aerial mycelium in group B isolates (No. HY03)
- (d) Moderate to dense aerial mycelium in Group D isolates (No. 23B)
- (e) Moderate to dense aerial mycelium in Group E isolates (No. 152H)



mycelium unlike the isolates from Sri Lanka where some isolates had no aerial mycelium while others had varying amounts of it.

3.1.4 Growth rate

The rate of growth on agar media varied between isolates and there was overlapping of values between the groups. This criterion was therefore, not of much value in delineating the groups. Growth between the first and the 4th day overlapped in isolates of groups A to E (Table 7).

3.1.5 Sporulation, zoospore characteristics and pore size of sporangia.

Some representative isolates from each group were examined for other criteria such as number of sporangia produced on agar medium, zoospores per sporangium, zoospore size and pore size of sporangium. On the basis of production of sporangia, it was possible to separate clearly Group B isolates from the rest of the isolates. These had a remarkable capacity for sporulation on agar (Table 8). In some isolates of Group B, clumping of sporangia was visible to the naked eye (Fig. 12a) and in some, clumps of sporangia gave the appearance of concentric rings (Fig. 12b). When viewed under the microscope, clumps of sporangia were seen as in Fig. 12c. Isolates in Groups A and C produced only a few sporangia whereas those in Groups D and E had a moderate number. In addition to this, the number of zoospores per sporangium and the size of the zoospore clearly separated Group B from other groups. The number of zoospores per sporangium was remarkably low in Group B, the mean values varied between 4.2 to 5.5, and the individual isolates varied from 1 - 12 zoospores per sporangium (Table 9; Fig. 12d). A characteristic feature in these isolates was that, very often, the contents of the sporangium emptied into a 2nd sporangium (Fig. 12e).

The size of the zoospore was larger in these isolates than in others, and the mean values varied between 11.9 - 12.1 μm (Table 10). The

Table 6. Colony morphology (aerial mycelium) of Phytophthora isolates from rubber

	Isolate no.		Aerial mycelium score ^a
Group A	1145, 1337, 1350, 1440	)	-
	1072, 1083, 1093	)	+
	1015, 1439	)	+/++
	1200, 1321	)	++
	1071	)	++/+++
	1339, 1438	)	+++
Group B	1117, 1223, 1303, 1334, TRT1, HYN2T	))	++
	1075, HY03	)	++/+++
Group C	3L, 5P, 5L, 365	)	-
	6P, 7P, 11P, 11L, 12B, 13B	)	
	16L, 17P, 20P, 21L, 22P, 22L	)	+
	23P, 24P, 26S, 27L, 29B, 361	)	
	434, 441		
	1P, 2P, 4P, 4L, 9P, 16P	)	+/++
	18P, 18L, 26L, 462	)	
	14P, 21P, 25P, 25L, 28P	)	
Group D	362, 444, 445, 456, 103	)	++
	105, 100, 109	)	
	81	)	+++
	23B, 51B, 1, 2, 3, 4, NS1, NS2, NS4, NS5	))	++/+++
Group E	8220	)	+/++
	152H, PP27, 15IC, 27IC, 53IC PPI	)	++/+++

^a Based on growth on LBA after 6 days at 25°C in the dark.
Assessed on scale:- none; + sparse; +/++ sparse but some in the region of inoculum; ++ moderate; ++/+++ moderate to dense, patchy; +++ dense

Table 7. Growth rate of Phytophthora isolates from rubber.

Mean growth between 1-4 day ^a (mm)	Isolate no.	Mean growth between 1-4 day ^a (mm)	Isolate no.
Group A			
35	1071	46	1072,1200,1350
41	1339	47	1337,1083
43	1438,1093,1145	50	1439
44	1015,1321	52	1440
Group B			
34	1117	47	1075,HYN2T
43	1303	50	HYO3
46	1212	54	TRT1
Group C			
29	29B	38	14P,26S,1P,100, 5P
30	361,23P	39	11P,3L,28P
31	25P	40	16L,9P
33	462,362,27L,441	42	13B,16P,12B,2P, 109
34	22L,6P,445,456, 22P,21P,21L	43	5L,7P
35	26L,25L,24P,4P	44	105,126
36	4L,444,20P	47	365
37	434,18P,11L,18L	52	1334,17P,81
		57	103
Group D			
42	1,23B	44	NS2,3,4,NS5
43	2,NS4,NS1	45	51B
Group E			
35	27IC		
41	PP27,152H,PPI		
44	15IC,		
46	53IC		
51	8220		

^a Each value is a mean of 3 replicates.

Based on growth on LBA at 25°C in light (12h) and dark (12h).

Table 8. Sporulation of selected Phytophthora isolates from rubber, on LBA at 25°C in light (12h) and dark (12h).

Mean sporangia no. ^a (mm ²)	Isolate no.
Group A	
18.2	1339
15.4	1439
18.1	1440
Group B	
283.1	TRT1
222.6	1334
202.5	HYN2T
Group C	
20.4	362
15.8	7P
14.6	6P
Group D	
82.4	51B
83.4	NS5
92.7	23B
Group E	
80.4	152H
76.5	PP27
64.3	151C

^a Each value is a mean of 54 observations.

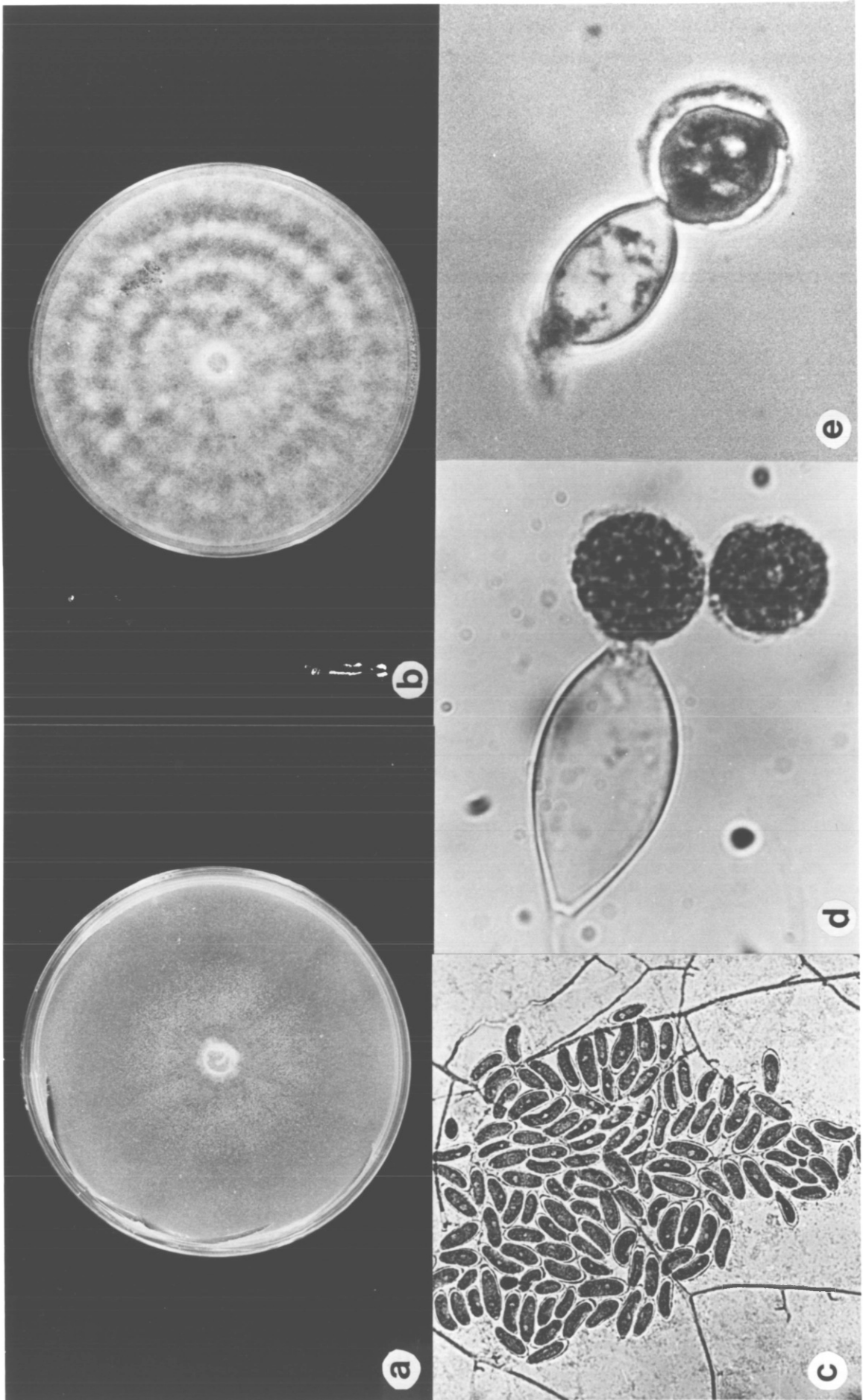
Table 9. Number of zoospores per sporangium of selected Phytophthora isolates from rubber

Mean No. ^a	Range	Isolate No.	Mean No. ^a	Range	Isolate No.
Group A					
29.1	(23-36)	1339	29.8	(25-37)	1083
29.4	(26-35)	1439	30.0	(25-35)	1440
Group B					
4.2	(1-10)	1075	4.4	(1-12)	HYN2T
4.4	(1-12)	1334	5.5	(1-12)	1117
Group C					
35.4	(30-38)	103	37.1	(35-40)	109
36.2	(35-38)	126	36.4	(35-40)	105
Group D					
29.9	(27-35)	NS2	30.6	(28-35)	23B
30.3	(24-34)	NS5	31.4	(28-32)	51B
Group E					
23.8	(17-35)	PP27	26.4	(18-33)	8221
24.1	(17-35)	PPI	28.5	(21-38)	152H

^a Mean of 20 observations.

Fig. 12 a,b,c,d & e

- (a) Clumping of sporangia in Group B Isolate no. 1334
- (b) Clumping of sporangia giving the appearance of concentric rings
- (c) Clumping as seen under the microscope
- (d) Zoospore release in group B isolates
- (e) Sporangium giving rise to a single secondary sporangium in Group B isolates



above characters did not provide firm evidence to support the separation of isolates into groups A, C, D and E, although Group C isolates generally produced a higher number of zoospores per sporangium. The pore size of sporangia was observed to be a less variable character in Groups A, B, C, D and E. Group B isolates generally had a slightly smaller pore size (Table 11).

3.1.6 Chlamydospore production and their size.

The production of abundant chlamydospores at temperatures suitable for production of sporangia (25°C) was characteristic of Group A isolates. These were mainly terminally attached on the hyphae but some were laterally placed. The chlamydospores were large with a mean diameter of $28.6\text{ }\mu\text{m}$ and the individual values varied between $20 - 37.5\text{ }\mu\text{m}$ (Table 12). Isolates in Group B, C and E did not produce chlamydospores at 25°C although a few were produced at lower temperatures of $18^{\circ} - 20^{\circ}\text{C}$, in some isolates. Chlamydospores were not produced in Group D isolates even at low temperatures. The chlamydospore sizes at a temperature of 20°C showed that the mean values for Group B isolates were small compared to other groups.

3.1.7 Pairing of isolates

Pairing studies of isolates showed that it was possible to produce oogonia and amphigynous antheridia when pairing was done between groups provided the right compatibility type was maintained. Similar observations were made when pairing was done within a group. The sizes of oogonia produced by different groups when paired with a suitable compatibility type isolate of Group C, is given in Table 13. This showed that Group B isolates produced the smallest oogonia. The mean size of oogonia for the five isolates examined varied between $19.4\text{ }\mu\text{m}$ to $22.0\text{ }\mu\text{m}$. This was however, not very impressive as the range of variation ($15\text{ }\mu\text{m} - 27.5\text{ }\mu\text{m}$) for these pairings sometimes overlapped with

Table 10. Size of zoospores of selected Phytophthora isolates from rubber.

Mean Size ^a (μm)	Isolate No.	Mean Size ^a (μm)	Isolate No.
Group A			
9.0	1440	9.4	1339
9.0	1439	9.7	1083
Group B			
11.9	1117	12.0	HYNT
12.0	HY03	12.1	1075
Group C			
8.6	105	10.6	109
10.6	126	10.7	103
Group D			
8.9	23B	9.5	NS2
9.5	51B	9.7	NS5
Group E			
9.3	27IC	9.5	152H
9.4	15IC	9.8	PP27

^a Each value is a mean of 50 observations

Table 11. Pore size of sporangia of selected Phytophthora isolates from rubber.

	Mean Pore Size (μm) ^a	Range (μm)	Isolate No.
Group A	7.8	7.5 - 8.75	1440
Group B	5.4	5.0 - 6.87	1117
Group C	7.75	7.5 - 8.75	126
Group D	8.0	7.5 - 8.75	NS2
Group E	7.5	7.5	152H
	7.25	6.25 - 7.25	8221
	7.37	6.25 - 7.25	PP27

^a Each value is a mean of 10 observations.

Table 12. Size of chlamydospores produced by selected isolates of Phytophthora from rubber.

Diameter (μm)		Isolate
Mean ^a	Range	No.
Group A		
26.3	20.0 - 35.0	1337
28.1	20.0 - 37.5	1350
28.1	22.5 - 37.5	1200
29.8	23.7 - 37.5	1093
30.6	23.7 - 37.5	1083
Group B		
25.7	20.0 - 32.5	1303
25.8	20.0 - 32.5	1223
25.9	20.0 - 32.5	1334
26.5	20.0 - 35.0	1117
27.9	20.0 - 32.5	1075
Group C		
30.5	25.0 - 36.2	126
31.3	25.0 - 37.5	17P
32.4	25.0 - 37.5	27L
32.4	25.0 - 37.5	9P
33.3	25.0 - 37.5	16P
33.4	27.5 - 37.5	13B
34.2	30.0 - 38.7	14P
Group E		
27.5	17.5 - 37.5	PP27
30.0	23.7 - 36.2	152H
30.0	23.7 - 35.0	15IC
30.3	25.0 - 36.2	27IC
31.6	25.0 - 36.2	53IC

^a Based on measurements of 50 chlamydospores from cultures grown on V8 agar at 18°C for 2 weeks.

the individual values in other groups, particularly when Group C was paired with Group A isolates.

3.1.8 Homothallic isolate from rubber.

The presence of abundant oogonia both in the host (Fig. 13a) and in culture (Fig. 13b) and paragynous Antheridia allowed isolate PO to be placed either in Group I, III or V of the key of Newhook et al. (1978).

Sporangia were generally not formed when isolate PO was grown on V₈ agar or LBA but on exposure to continuous light at 25°C for approximately 4-5 days, some semi-papillate sporangia were formed. They were irregular (Fig. 13c,d,e & f) with a mean size of (40.0µm-65µm) 52.6 µm x 33.9µm (28.75µm - 45.0µm) and a mean L/B ratio of 1.5. Most sporangia had a truncate base. Sporangia of smaller size were formed when mycelial plugs were placed in sterile distilled water. The pore size of the sporangia was less than 7.5µm. The sporangia were non-caducous and non-proliferating. These features indicated that the isolate belonged in Group III of Newhook et al (1978). The presence of irregular sporangia and abundant oogonia in cultures also agree with the description given for P. citricola. Oogonia were spherical, 27µm - 37.5 µm. Oospores were generally thick-walled and plerotic.

Although sporangia of P. citricola are generally known to have more than one apex, this character was not prominent in the isolate PO. The absence of broadly ovoid sporangia and catenulate hyphal swellings on the mycelium aided in separating this from P. syringae which is also in the Group III. It differed from P. porri in the same group which is known to have a very slow growth and produce hyphal coils on agar. It also differed from P. primulae, due to the absence of very elongated sporangia often in series.

Table 13. Size of oogonia produced by selected Phytophthora isolates
from rubber when paired with two isolates (Nos. 126 or 362)
from Group C

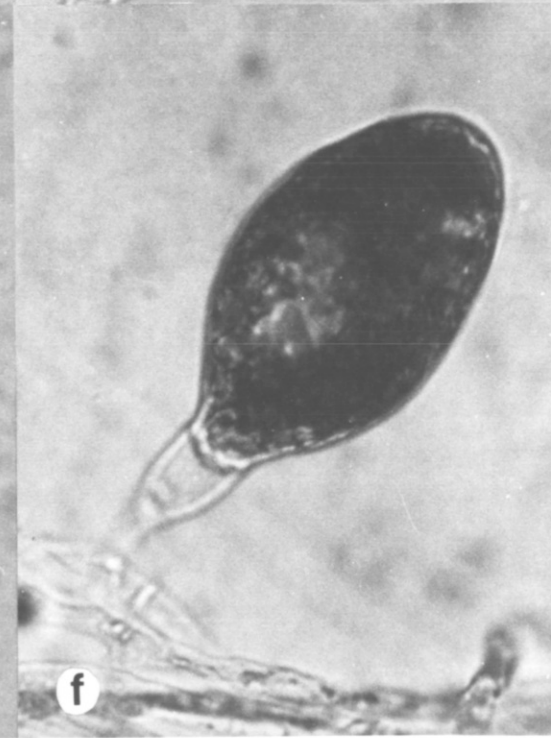
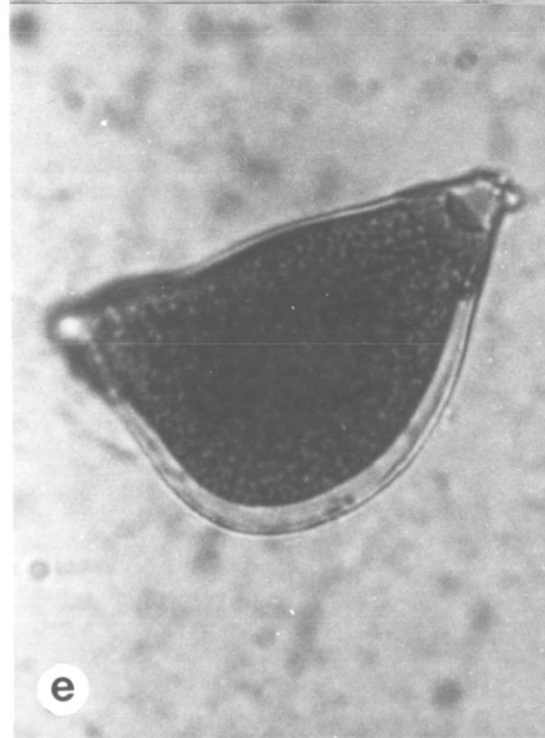
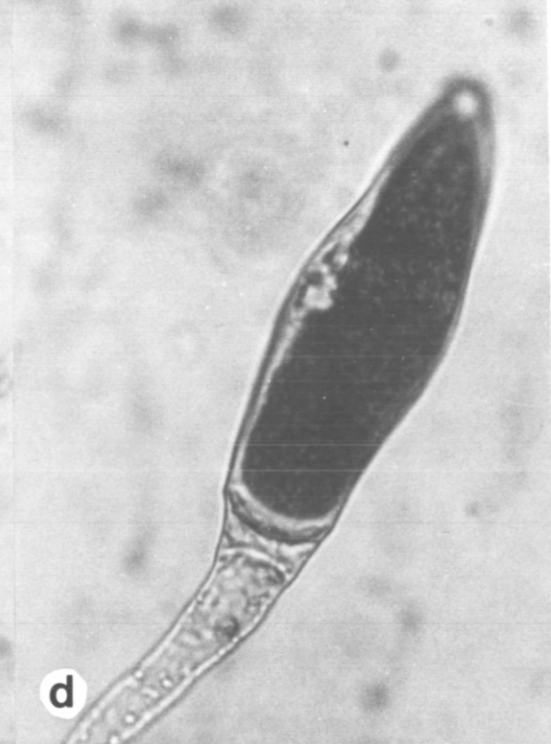
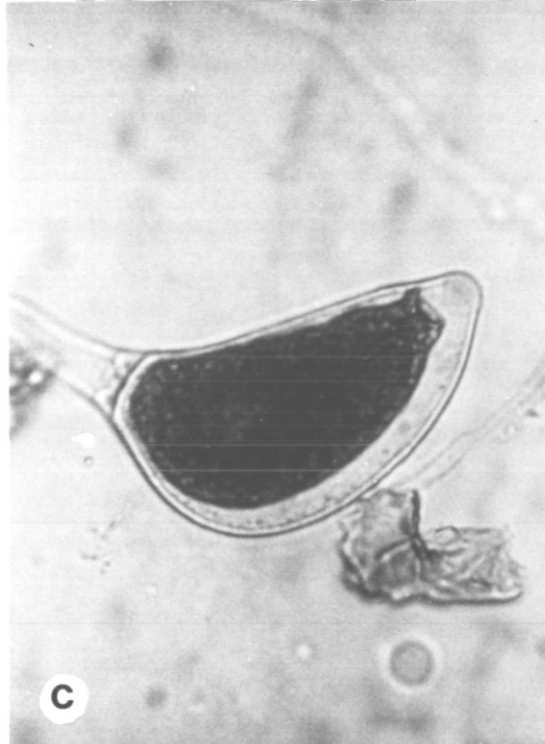
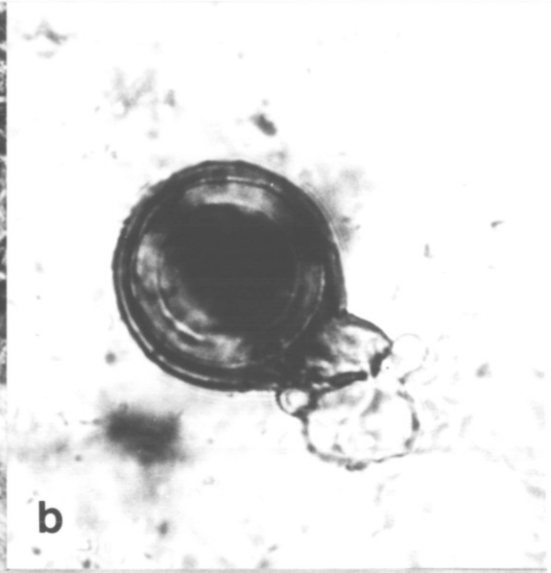
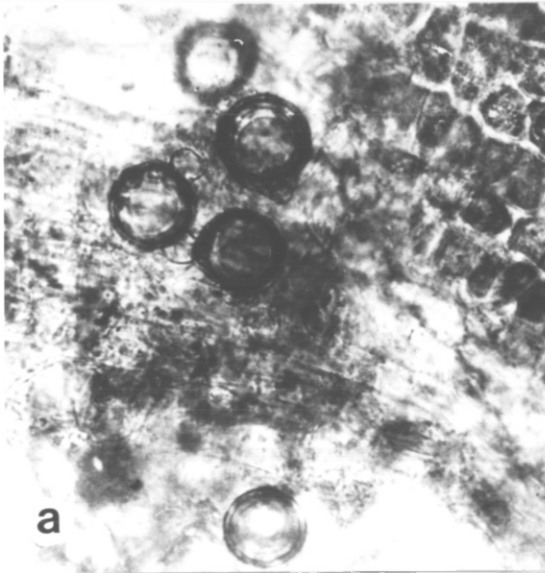
Pairings	Mean diameter ^a (μ m)	Range (μ m)
Group A		
1438 x 126	22.6	20.0 - 25.0
1083 x 362	23.8	20.0 - 26.2
Group B		
1223 x 126	19.4	15.0 - 22.5
1334 x 126	21.0	15.0 - 27.5
1117 x 126	22.0	17.5 - 25.0
1303 x 126	19.9	17.5 - 22.5
HYO3 x 362	20.6	17.5 - 22.5
Group C		
6P x 126	22.3	17.5 - 25.0
5P x 126	23.3	18.75 - 27.5
3L x 126	24.3	20.0 - 30.0
4P x 126	26.9	21.2 - 32.5
105 ^b x 126	29.7	27.5 - 35.0
81 ^b x 362	31.8	27.5 - 35.0
Group D		
1 x 126	24.3	21.0 - 27.5
2 x 126	26.5	21.2 - 35.0
NS4 x 126	26.9	18.7 - 30.0
NS5 x 126	27.3	20.0 - 32.5
51B x 126	27.5	23.7 - 30.0
Group E		
PPI x 362	27.9	23.7 - 32.5
53IC x 362	28.4	25.0 - 32.5
15IC x 362	30.9	27.5 - 37.5
8221 x 126	29.3	27.5 - 37.5

^a Each value is a mean of 25 observations

^b Isolates from India.

Fig. 13 a,b,c,d,e & f

- (a) Sex organs of Isolate P0 on petiolar tissue
- (b) Oogonium and perigynous antheridium of Isolate P0
- (c),(d),(e),& (f) Shapes of sporangia in Isolate P0



3.1.9 Identification of species

With the different criteria examined it was possible to delineate clearly the isolates into groups A, B, C, D and E. Group A isolates resembled P. palmivora morphological form 1 in pedicel length, occluded nature of the pedicel, caducous sporangia with short (5µm) and broad pedicel as given in the key of Newhook et al. (1978). Group B isolates were identified as P. botryosa with its characteristic clumped sporangia, which are elongated and caducous with a slender pedicel of length less than 10µm. Group C isolates corresponded to P. meadii in the above key and had variable shaped sporangia which were caducous, with a pedicel length of approximately 10-20µm. Group D was clearly designated as P. palmivora morphological form 4 (hereafter referred to as MF₄) with its long pedicel, caducous sporangia, having an elongated shape with a tapered base. In addition to these characters, the presence of a cross wall near the base of the sporangium served as a useful diagnostic feature. Group E isolates belong to P. citrophthora, with variable shaped sporangia, often with more than one apex. Although sex organs are not formed in single or paired cultures of P. citrophthora, according to the descriptions given in the key of Newhook et al. (1978), Group E isolates produced sex organs when paired with Group C isolates (P. meadii) of compatibility type A₁. Based on the morphological characters it was possible to place isolate P0 under P. citricola (D.J. Stamps, Personal Communication). These studies show that the species of Phytophthora causing disease in the rubber tree vary in different countries where rubber is grown. These are indicated in Table 14.

Table 14. Summary of distribution of Phytophthora species in eight countries

Country	Species	Compatibility type
Brazil	<u>P. palmivora</u> (MF ₄)	A ₁
China	<u>P. citrophthora</u>	A ₁
	<u>P. palmivora</u>	A ₂
India	<u>P. meadii</u>	A ₁ & A ₂
Indonesia	<u>P. citrophthora</u>	A ₂
	<u>P. palmivora</u>	A ₂
Ivory Coast	<u>P. citrophthora</u>	A ₂
Malaysia	<u>P. palmivora</u>	A ₁ & A ₂
	<u>P. botryosa</u>	A ₂
Sri Lanka	<u>P. meadii</u>	A ₁ & (A ₂ = 1 isolate only)
	<u>P. citricola</u>	Homothallic
Thailand	<u>P. botryosa</u>	A ₁ & A ₂

3.2 Isolates from cocoa

3.2.1 Morphological characters

Except for one isolate (isolate no. PC), all isolates had distinctly papillate sporangia and they produced amphigynous oogonia in dual cultures. They were thus assigned to group II in the key of Newhook et al. (1978).

These isolates produced caducous sporangia and on the basis of pedicel length these were assigned to one group. They had distinctly short pedicels with a mean pedicel length varying between 1.91 μ m to 3.05 μ m and most individual isolates varied between 1.25 μ m to 5.0 μ m (Table 15). Isolate no. PC produced a large number of oogonia with amphigynous antheridia, on agar media, both at 25° and 28°C.

Presence of dense aerial mycelium was characteristic of cocoa isolates, with isolate no. DIN4 having slightly more aerial mycelium than others (Table 16). Isolates were either faintly stellate, stellate or without any stellate pattern. Isolate PC did not have aerial mycelium or petaloid character.

The L/B ratio of isolates (except PC) varied between 1.13 μ m to 1.81 μ m which showed that there was greater variability in shape (Table 17). Isolate 26B had elongated sporangia while those of isolates DIN4 and 404 were almost spherical in shape; their mean L/B ratio being 1.81, 1.13 and 1.23, respectively. Their individual values varied between 1.03 - 2.18.

Isolate PC did not produce sporangia on agar media usually, but produced only a few, occasionally, and it was insufficient for a study on morphological characters of sporangia. However, when agar plugs containing mycelia and oospores were placed in distilled water at 25° and 28°C, sporangia were produced in large numbers. Sporangia thus produced were small, predominantly papillate, occasionally bi-papillate,

irregular in shape and varied from spherical, ovoid or obturbinate (Fig. 14a, b, c). Occasionally, intercalary sporangia were also seen. Although sporangia were deciduous they did not shed easily. The pedicel length varied between $10\mu\text{m}$ - $27\mu\text{m}$ and often had swellings on them (Fig. 14d).

The mean incremental growth between 1st and 4th day for most heterothallic isolates was consistent and varied between 53.5mm to 59.5mm. The homothallic isolate PC was slow in growth. This grew only 43mm up to the 4th day, the incremental growth being only 29mm (Table 18).

Representative isolates were examined for other characters such as sporulation, zoospore number per sporangium, zoospore size and pore size. Their values are given in Table 19. All these characters did not vary markedly in the isolates examined. They all belonged to compatibility type A_2 . A large number of chlamydospores were produced at 25° and 28°C in all isolates except isolate PC. They were mostly terminal and mean size varied between $29.3\mu\text{m}$ - $32.2\mu\text{m}$ (Table 20). Some chlamydospores were large and varied in size between $38\mu\text{m}$ - $40\mu\text{m}$.

Antheridia in isolate PC were predominantly amphigynous, small and somewhat spherical (mean diameter $10\mu\text{m} \pm 1.30$ x $9.95\mu\text{m} \pm 0.90$) and were one-celled. A characteristic feature in this isolate was the ornamentation of the oogonial wall. About 4-6 projections were seen in all mature oogonia (Fig. 15). Young oogonia lacked this ornamentation (Fig. 16). Oogonia were characteristically long, uniform in shape and had a tapered base. The mean diameter was $21.43\mu\text{m}$ (Range $18.75\mu\text{m}$ - $24.38\mu\text{m}$) and mean oospore size was $19.72\mu\text{m}$ (Range $16.8\mu\text{m}$ - $21.88\mu\text{m}$).

They were mostly thick-walled, aplerotic and occasionally plerotic. Based on the above features isolate PC was identified as P. katsurae. All the other isolates on cocoa conformed to the description given for

Table 15. Pedicel length of Phytophthora isolates from cocoa

Pedicel length ^a Mean (μm)	Range (μm)	Isolate No.
1.91	0.62 - 5.0	DIN4
2.01	0.75 - 3.75	M7
2.02	1.25 - 2.5	M4
2.24	1.25 - 3.75	447
2.34	1.25 - 3.75	K3
2.40	1.25 - 3.75	T1
2.44	1.25 - 3.75	404
2.49	1.25 - 5.0	26B
2.61	1.25 - 5.0	Y3
2.74	1.25 - 5.0	K11
2.81	1.25 - 5.0	Y14
3.05	1.25 - 5.0	Y20

^a Each value is a mean of 50 observations.

Table 16. Colony morphology of Phytophthora isolates from cocoa.

Isolate No.	Petaloid character ^a	Aerial mycelium ^b score
PC	Uniform	-
DIN4	"	+++
404	"	++/+++
447	"	++/+++
Y20	"	++/+++
M4	"	++/+++
M7	Faintly stellate	++/+++
Y14	" "	++/+++
26B	" "	++/+++
K3	" "	++/+++
T1	Stellate	++/+++
K11	"	++/+++

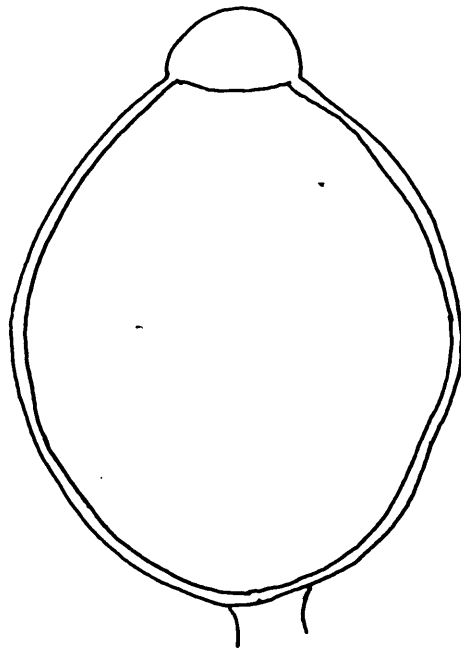
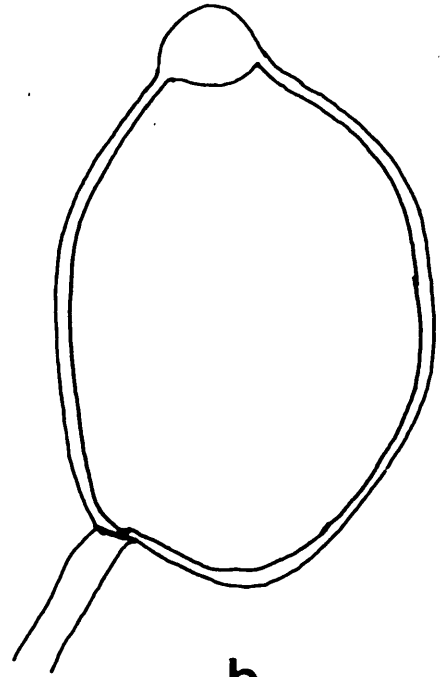
^a Based on growth on LBA (after 6 days at 25°C in the dark. Assessed as uniform (petaloid character absent), faintly stellate, stellate, distinctly stellate.

^b Assessed on scale:- None; + sparse; ++/++ sparse but some in the region of inoculum; ++ moderate; ++/+++ moderate to dense, patchy; +++ dense.

Fig. 14 a,b,c & d

(a,b & c) Sporangial shapes of Isolate no. PC

(d) Swelling on pedicel of Isolate no. PC

**a****b**

25 μm

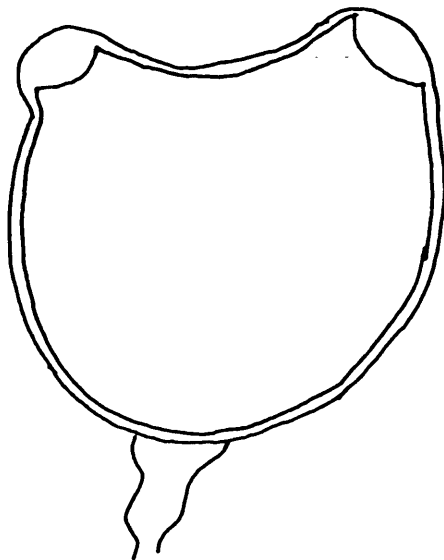
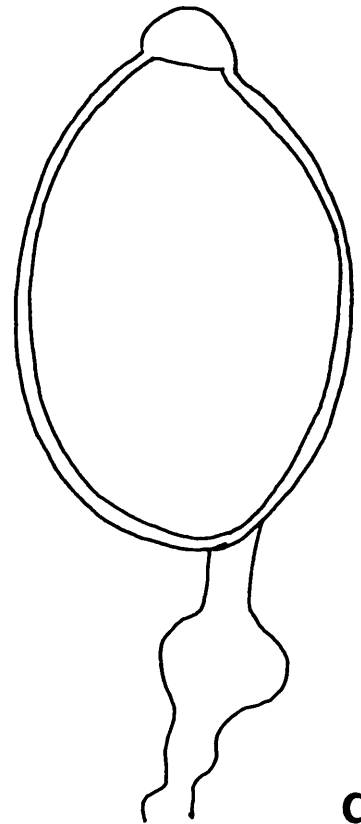
**c****d**

Table 17. Dimensions of sporangia of Phytophthora isolates from cocoa.

Mean L/B ratio ^a	Mean length (μ m)	Mean breadth (μ m)	Isolate No.
1.13	34.15	30.27	404
1.23	39.85	32.33	DIN4
1.29	28.8	22.4	PC
1.33	41.22	31.1	K3
1.34	39.35	29.9	Y20
1.39	46.27	34.4	M7
1.40	42.3	30.65	Y14
1.43	39.35	27.8	Y3
1.44	40.07	28.17	K11
1.48	43.29	29.25	447
1.57	43.12	27.8	T1
1.62	42.77	26.1	M4
1.81	45.05	24.89	26B

^a Each value is a mean of 50 observations.

Table 18. Growth rate of Phytophthora isolates from cocoa

Isolate	Growth between 1-4 day ^a	Mean
No.	Mean incremental growth (mm)	growth (mm)
PC	29	43
M4	46	63
M7	46	65
DIN4	53	78
447	55	75
404	55	75
K11	55	75
T1	55	75
Y20	55	75
Y3	56	77
K3	58	78
Y14	59	77
26B	59	79

^a Each value is a mean of 3 replicates

Based on growth on LBA at 25°C in light (12h) and dark (12h)

Table 19. Sporulation and characteristics of zoospores and zoospore release in Phytophthora isolates from cocoa.

Isolate No.	Mean ^a	Zoospore size (μm)	No. zoospores/sporangium
	No. sporangia/mm ²		
DIN4	39.2	9.76	27.1
26B	37.9	9.20	26.3
Y14	28.9	9.17	26.5
Y20	21.7	9.45	27.3
M4	20.3	9.65	27.3
M7	20.1	9.04	26.8

^a Based on isolates grown on LBA at 25°C in light (12h) and dark (12h).

Table 20. Size of chlamydospores produced by isolates of Phytophthora from cocoa.

Isolate No.	Diameter (μm)	
	Mean ^a	Range
K3	29.3	25.0 - 36.2
Y3	29.4	25.0 - 35.0
447	31.0	25.0 - 37.5
M4	31.7	26.2 - 38.7
404	32.2	25.0 - 40.0

^a Based on measurements of 50 chlamydospores from cultures grown on V8 agar at 18°C for 2 weeks.



Fig. 15. Mature sex organs in Isolate PC
Note protruberances on oogonial wall

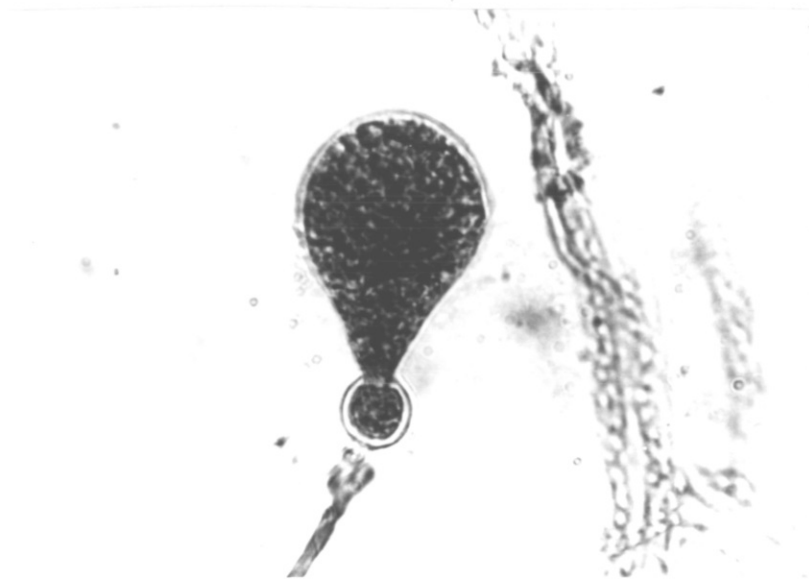


Fig. 16. Immature sex organs in Isolate PC

P. palmivora in the key of Newhook et al. (1978).

3.2.2 Abnormal oogonia in pairings of P. palmivora isolates from cocoa and P. meadii isolates from rubber.

Several abnormal oogonia were observed when Phytophthora isolates from cocoa and rubber were paired. A separate study was therefore, carried out to determine the percentage abnormal/aborted oogonia in several pairings. It was observed that the number of oogonia produced in the inter-specific pairings is generally low, the values varied between $14.2 - 25.0/\text{mm}^2$ compared to $51.6/\text{mm}^2$ for a pairing of compatible isolates from rubber. Percentage abnormal oogonia varied between 18.2-32.3% (Table 21). In most pairings, ^{the} antheridium appeared to be fully formed but the oogonium did not develop to reach the normal size (Fig. 17a,b,c & d). The size of the normal oogonia was small (Table 22).

3.2.3 Inoculation of rubber petioles with Phytophthora species.

When rubber petioles of four clones were inoculated with different species of Phytophthora from rubber and one from cocoa, highly significant differences were observed between isolates and clones. Overall, clones PB 86, RRIC 103, RRIC 121 were most susceptible to the species tested, with RRIC 110 and RRIC 100 being somewhat tolerant. The isolate x clone interaction was also highly significant, indicating that isolates behave differently on the 5 clones. P. citrophthora and MF₄ isolates caused the largest lesions and their reaction on petioles of each clone tested was similar with no significant differences between them. P. palmivora (R) and P. palmivora (C) isolate from rubber and cocoa, respectively, showed a similar reaction on clones RRIC 121, RRIC 103 and RRIC 110 but isolate of P. palmivora (C) was less pathogenic than P. palmivora (R) on clones RRIC 100 and PB 86. The latter was most susceptible to P. meadii and the former was least susceptible. A similar pattern was observed with P. palmivora (R) isolate (Table 23).

Table 21. Percent abnormal oogonia in pairings of P. palmivora isolates from cocoa with a compatible isolate of P. meadii from rubber.

Pairings ^b	Oogonia per mm ²	Abnormal ^a oogonia (%)
Y20 x 9P	14.2	32.3
Y3 x 9P	16.9	21.4
Y14 x 9P	20.5	24.7
K11 x 9P	17.0	24.4
K3 x 9P	15.9	22.2
T1 x 9P	15.8	23.7
404 x 9P	25.0	18.2
447 x 9P	17.4	22.8
M4 x 9P	19.8	25.3
M7 x 9P	14.3	22.9
126 x 9P ^c	51.6	0

^aExpressed as a percentage of the total number of oogonia

^bAll isolates paired with isolate 9P from rubber

^cPairing of compatible isolates from rubber

Fig. 17. a,b,c & d. Abnormal sex organs in pairings of P. palmivora isolates from cocoa with a P. meadii isolate from rubber.

- (a) Normal and abnormal oogonia (Isolate Y20x9P)
- (b) Normal oogonium; well developed antheridium but oogonium not developed (Isolate Y20x9P)
- (c) Partly formed sex organs (Isolate K11x9P)
- (d) Partly formed antheridia, oogonia not developed (Isolate T1x9P).

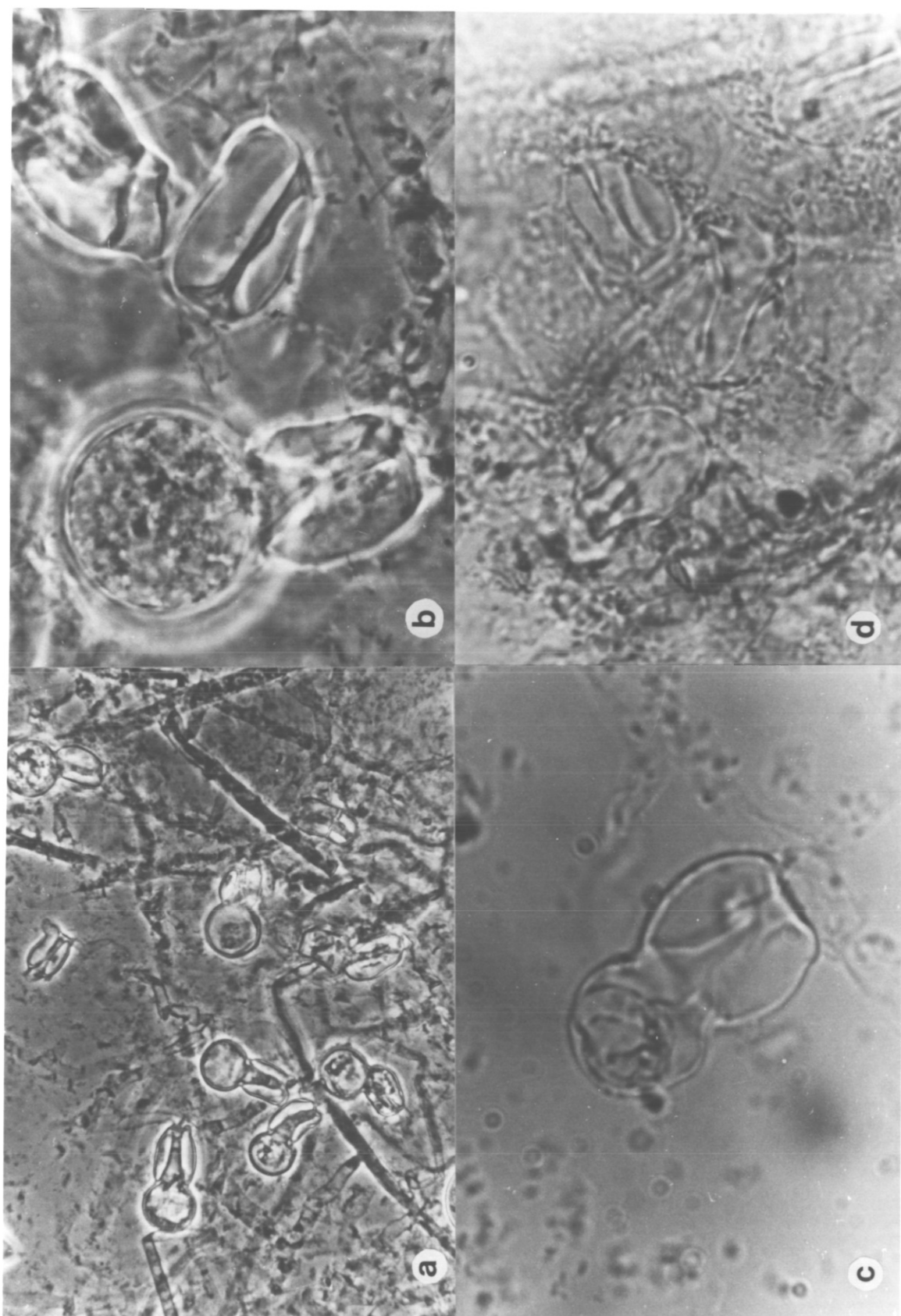


Table 22. Size of oogonia in pairings of P. palmivora isolates from cocoa with a compatible isolate of P. meadii from rubber

Pairings	Mean diameter ^a (μ m)	Range (μ m)
K11 x 9P	17.8	12.5-20.0
404 x 9P	17.8	15.0-20.0
K3 x 9P	18.0	12.0-23.7
447 x 9P	18.1	15.0-20.0
Y14 x 9P	18.3	15.0-22.5
M4 x 9P	18.4	15.0-22.5
Y3 x 9P	18.5	12.5-20.0
T1 x 9P	19.0	17.5-22.5
M7 x 9P	19.2	17.5-20.0
Y20 x 9P	19.2	15.0-22.5

^a Based on measurements of 25 oogonia

Overall, P. meadii has similar reactions to P. palmivora (R) on all clones. The lesions caused by P. botryosa on clone RRIC 121 and RRIC 103 were significantly longer than that produced by P. meadii but this effect was not seen on other clones. P. citricola was the least active in inducing lesions but was as effective in doing so as P. palmivora (C) except on clone RRIC 103.

3.2.4 All isolates of Phytophthora species from rubber and cocoa tested here, were pathogenic to pods of Amelonado cocoa in varying degrees (Table 24). The results indicate that on the ability to infect cocoa pods the isolates could be placed in several groupings. Isolates from rubber, P. citrophthora and P. palmivora from Indonesia and MF₄ from Brazil were most pathogenic. The two groups (15IC, 362, AC8220 from rubber and Y3 and P0 from cocoa were moderately pathogenic. It was also observed that isolate P0 (P. citricola) obtained from rubber was more pathogenic to cocoa than to rubber. P. botryosa (Isolate TRT₁) and P. meadii (A₂ type) were relatively weak pathogens while P. katsurae from Ivory Coast was virtually non-pathogenic.

Table 23 Mean lesion length on rubber clones caused by species of Phytophthora

Species	Mean lesion length (mm)					Total lesion length (mm)
	RRIC 121	RRIC 103	Clone PB 86	RRIC 110	RRIC 100	
<u>P. citophthora</u>	315	296	302	231	196	1340
MF ₄	293	268	302	236	205	1304
<u>P. botryosa</u>	272	262	265	219	186	1204
<u>P. meadii</u>	222	207	241	221	189	1080
<u>P. palmivora</u>	206	207	238	192	183	1026
<u>P. palmivora</u> ^a	201	208	185	182	152	928
<u>P. citricola</u>	190	179	167	169	143	848

^a P. palmivora isolate from cocoa.

Table 23 (continued)

	D.f.	S.S.	M.S.	F
Isolates	6	13924.493	2320.75	75.08***
Clones	4	7055.5219	1763.88	57.07***
Isolates x Clones	24	2626.7451	109.45	3.54***
Error	70	2164.0	30.91	-

Total 104 25770.76

S.E. for isolate totals = 21.5

S.E. for clone totals = 25.5

S.E. for clone x isolate = 19

	<u>Isolates</u>		<u>Clones</u>
<u>P. citrophthora</u>	1340 a	PB86	1700 a
MF ₄	1304 a	RRIC 121	1699 a
<u>P. botryosa</u>	1204 b	RRIC 103	1627 b
<u>P. meadii</u>	1080 c	RRIC 110	1450 c
<u>P. palmivora</u>	1026 d	RRIC 100	1254 d
<u>P. palmivora</u> (c)	928 e		
<u>P. citricola</u>	843 f		

Table 24. Lesion size on Amelonado cocoa pods caused by Phytophthora species.

Isolate no.	Species	Lesion size (cm ²)			Mean lesion size (cm ²)
AC8220	<u>P. citrophthora</u>	30	32	28	30.0
15IC	<u>P. citrophthora</u>	33	31	30	31.3
PPI	<u>P. citrophthora</u>	39	36	33	36.0
362	<u>P. meadii</u> (A ₁ type)	31	33	28	30.7
126	<u>P. meadii</u> (A ₂ type)	13	12	12	12.3
PPII	<u>P. palmivora</u>	37	40	40	39.0
TRT ₁	<u>P. botryosa</u>	18	19	21	19.3
155	MF ₄	40	39	32	37.0
PO	<u>P. citricola</u>	28	24	23	25.0
Y3	<u>P. palmivora</u> ^a	28	27	27	27.3
PC	<u>P. katsurae</u> ^a	2	2	2	2.0

^a Isolates from cocoa

	D.f	S.S.	M.S.	F
Isolates	10	3794.30	379.43	77.75
Error	22	107.34	4.88	
Total	32	3901.64		

C.F. = 22936.364

S.E. = 1.3

PPII	155	PP1	15IC	362	AC8220	Y3	PO	TRT ₁	126	PC
<u>39.0</u>	<u>37.0</u>	<u>36.0</u>	<u>31.3</u>	<u>30.7</u>	<u>30.0</u>	<u>27.3</u>	<u>25.0</u>	<u>19.3</u>	<u>12.3</u>	<u>2.0</u>

Discussion

Taxonomic studies have provided much information on variability within and amongst Phytophthora species. Although new criteria such as protein and enzyme patterns, serology, chromosome number help us to understand taxonomic relationships amongst species, morphology still provides the primary basis for identification (Zentmyer, 1980).

The Phytophthora isolates collected from different countries where rubber is grown, were separated into 5 species mainly on the basis of important morphological characters such as the papillate nature of the sporangium, presence or absence of sex organs in single culture, pedicel length, size and shape of sporangium and presence or absence of chlamydospores.

Of the several criteria examined the pedicel length proved to be the most useful. On this character alone, it was possible to distinguish 2 species (P. palmivora and MF₄). The characteristic short, broad pedicels of P. palmivora differed strikingly from the longer pedicels of MF₄, while the shape of the sporangium of MF₄, was another distinctive character.

Waterhouse (1974) emphasized the importance of caducity and pedicel length as a useful taxonomic character. Subsequently, Griffin (1977), Tsao & Tumakate (1977), Kaosiri et al. (1978) and Brasier & Griffin (1979) studying certain P. palmivora isolates from cocoa and black pepper also recognized the importance of caducity and pedicel length as diagnostic characters. However, pedicel length character is useful only when identifying species that readily shed their sporangia such as P. palmivora, P. botryosa and MF₄. In less caducous species (P. citrophthora) mechanical shaking to dehisce sporangia could result in damage to the pedicel thus giving erroneous results.

When a species cannot be identified by using pedicel length as the

main criterion, other characters have to be examined. Sporangial size and shape and L/B ratio have often been used as a taxonomic parameter in identifying particularly, P. palmivora (Chee, 1969 ; Waterhouse, 1974). In this study the size and/or shape of the sporangium was found to be useful in distinguishing three species, P. botryosa, P. citrophthora and MF₄. Isolates of P. botryosa had smaller sporangia than those of other species, particularly in respect of sporangium breadth. The sporangia of P. citrophthora were of variable shape and also bi-papillate which separated them from most other isolates. The sporangium of MF₄ was boat shaped and had a characteristic truncate base.

The pattern of release of zoospores, zoospore number and size have not been hitherto used in the identification of Phytophthoras. In every species examined there was considerable variation in the number of zoospores produced within sporangia. However, P. botryosa was characteristically different to other species with a mean value of only 4-5 zoospores per sporangium (Table 9). P. meadii generally produced a larger number of zoospores per sporangium. The zoospore of P. botryosa also tended to be slightly larger than those of other species. A feature that was consistently observed in P. botryosa isolates was the emptying of the contents into a secondary sporangium. These characters helped further to distinguish P. botryosa from other species.

The presence of chlamydospores was specially useful in identifying P. palmivora isolates. This species generally produced chlamydospores in abundance under most conditions but specially at temperatures which favoured sporangial production (25° - 28°C). P. meadii rarely produced chlamydospores confirming the importance of this character in the separation of the two species as indicated by Waterhouse (1963).

Generally, isolates produced more aerial mycelium and colonies, if stellate at all, were more distinctly so on V₈ agar than on LBA.

Growth rate was generally too variable to be useful in separating species though slow growth was a feature of P. katsurae from cocoa (Table 18).

The presence of sex organs and the type of antheridium (paragynous or amphigynous) is a useful aid to classification of species (Waterhouse, 1963). P. citricola (isolate P0) from rubber and P. katsurae (isolate PC) from cocoa were easily separated from other species because they produced sex organs in single culture. In P. citricola the antheridium was paragynous whereas in P. katsurae it was amphigynous. Further, the distinct ornamentation of the oogonial wall clearly distinguished P. katsurae.

This study showed that there was considerable variation in morphological characters. Of those examined, pedicel length was most reliable and this character deserves closer attention from all workers when identifying an unknown isolate of Phytophthora.

One isolate collected in December 1985 from Dartonfield Estate of the RRISL, outside the normal disease period, was identified as P. citricola Sawada. This is the first record of this pathogen on rubber in any rubber growing country in the World. It is important that further surveys should be undertaken to examine the distribution of this species.

Of the 45 isolates from Sri Lanka, 36 were collected in 1983 and these belonged to P. meadii A₁ compatibility type. Among the 9 isolates that were collected in 1966 - 1972 and maintained at the Culture Collection of RRISL and used here, only one isolate was grouped under P. meadii A₂ compatibility type and the remainder were P. meadii A₁ compatibility type. Satchuthananthavale (1963) found both compatibility types in isolates collected from one locality with type A₂ predominating. She also found both compatibility types even from a

single tree. The absence of P. meadii A₂ type in the present collection of isolates from all areas where rubber is grown in Sri Lanka, shows that there has been a definite change in the type of pathogen present now. Peries and Dantanarayana (1966) reported that out of 14 isolates, 8 isolates were P. meadii homothallic and the remainder belonged to P. meadii A₂ compatibility type. The absence of homothallic P. meadii isolates in this study is notable. However, the presence of a single homothallic isolate (P. citricola) in the recent collection of isolates creates a doubt as to whether the homothallic isolate was mis-identified as P. meadii by the earlier workers possibly due to lack of taxonomical information. The clones planted in the recent times have changed from that of the 1960's. The P. meadii A₂ type and homothallic P. meadii isolates were obtained in the 1960's from clones such as LCB 870 and NAB2, NAB6 and NAB12. At present, these clones have been totally replaced by newer clones and therefore, there is a strong possibility that the pathogen type is now changed.

In Indonesia, the disease caused by Phytophthora was attributed to P. palmivora (Dijkman, 1951; Nara et al., 1968). Of the two isolates received, one from Central Java was identified as P. palmivora and the other, from North Sumatra, as P. citrophthora. This latter species has not been reported previously, to affect Hevea in Indonesia. Both isolates were of the A₂ compatibility type. As only two isolates were examined in this study it is possible that A₁ compatibility type of the above species as well as other species may be present if a large number of isolates are examined. It could be that in the two islands of Indonesia, Java and Sumatra, the predominant species are P. palmivora and P. citrophthora, respectively.

The causal organism of Phytophthora disease of rubber in Ivory Coast was unknown before this study was made. The five isolates collected

from widely different regions in Ivory Coast proved to be P. citrophthora, A₂ compatibility type. Formation of oogonia has not been reported for intra and inter-specific pairings of P. citrophthora isolates. However, four of the five isolates produced oogonia both in inter-specific as well as intra-specific pairings. Although this difference was observed all other characters agreed with those of P. citrophthora given in the key of Newhook et al. (1978). However, in view of this discrepancy, more isolates from Ivory Coast need to be examined.

Although Brasier and Griffin (1979) examined isolates of Phytophthora from cocoa in the Ivory Coast they did not find P. katsurae. The identification of isolate PC as this species, in this study thus constitutes a new record. Two more isolates received from The Ivory Coast after this investigation was virtually complete were also found to be P. katsurae, so this species may be fairly common in the Ivory Coast.

The identification of isolates from Thailand and Malaysia as P. botryosa confirmed the previous findings of Chee & Greenwood (1968) for Thailand and that of Chee (1969) for Malaysia. Both compatibility types were identified among the three isolates from Thailand included in the present study, which fact has not been previously observed. Although all five isolates from Malaysia were of the A₁ compatibility type the presence of both types have been reported previously (Tan et al., 1977). The occurrence of both strains gives the possibility of hybridization and the emergence of new strains. A similar situation can also develop in India where both compatibility types of P. meadii are found as shown in this study.

P. palmivora is reported as a pathogen in most countries where rubber is grown (Sri Lanka, Dantanarayana et al. (1984); Brazil, Madeiros

and Ayres, 1965; Malaysia, Chee et al. 1967; India, Thankamma, et al.; 1968; China, Ho et al., 1984; Indonesia, Nara et al.). The possibility that this isolate is also present in the Ivory Coast cannot be excluded until a detailed investigation is carried out with more isolates. P. botryosa is somewhat more restricted and occurs in Northern Malaysia, Thailand, China and the Andaman Islands in the Indian Ocean. It has not been reported outside this area. However, distribution of P. citrophthora occurs in countries which are wide apart namely China, Indonesia and Ivory Coast. MF₄ at present is confined only to Brazil in South America.

Although relatively few isolates were studied from rubber and cocoa four new records (P. citrophthora and P. katsurae in Ivory Coast, P. citrophthora in Indonesia, P. citricola in Sri Lanka) are reported here. This highlights the need to examine more isolates from some countries, especially Indonesia and the Ivory Coast. This is particularly important when there is an exchange of seeds and budwood of rubber and cocoa between countries, for breeding purposes because this increases the risk of introducing new species of Phytophthora. All species identified in this study were able to produce oospores, when pairing was done between species, thus producing sexual organs which can withstand unfavourable conditions and also produce new strains, if they germinate. Therefore, it is important to take proper precautionary measures such as treatment of plant material and growing them in quarantine before they are planted in the field.

The results of pathogenicity experiments (Tables 23 & 24) show that an isolate of P. palmivora from cocoa could infect petioles of currently grown clones of rubber. Similarly, the ability of several species of Phytophthora from rubber to cause infection of cocoa pods of the widely grown variety Amelonado was also demonstrated. This shows that it is

possible for cross infection to occur when rubber and cocoa are interplanted. Under Sri Lankan conditions, foci for inoculum build up take place on rubber pods (Peries, 1969). During heavy rains continuous dissemination of zoospores can lead to cocoa pod infection. It is also likely that inoculum from cocoa pods can get splashed-dispersed onto tapping panels of rubber. Therefore, caution has to be exercised in interplanting rubber and cocoa particularly in areas where the rainfall is high and spread out over a long period.

However, interspecific pairings of P. meadii isolates from rubber and P. palmivora isolates from cocoa produced 23% - 33% abortive oogonia (Table. 21). Even though nearly one-third of the oogonia are abnormal, the presence of a large number of normal oogonia indicates the high risk of interplanting cocoa with rubber, especially in wet areas. However, in dry areas where conditions are unfavourable for disease development, intercropping could be attempted.

CHAPTER 2

STUDIES ON PHYTOPHTHORA ISOLATES
FROM SRI LANKA

1. Introduction

Chlamydospores and oospores have been observed in mummified rubber pods and their stalks (Peries, 1964) but experimental evidence is lacking for their role in initiating infection.

P. meadii was identified as a homothallic species (McRae, 1918). Similar observations were made by Orellana (1959) and Turner (1961). The occurrence of two distinct compatible types of Phytophthoras affecting Hevea in Sri Lanka was shown by Satchuthananthavale in 1963. Peries and Dantanarayana (1965) showed that P. meadii in addition to being homothallic was sexually compatible with isolates of P. palmivora from rubber. Satchuthananthavale (1963) observed that members of one or the other compatibility group predominated in any particular location but Peries and Dantanarayana (1966) observed that they could occur even on the same tree.

Waterhouse (1974) noted that chlamydospores were rarely produced in culture by isolates of P. meadii. Similar observations were made by Dantanarayana et al. (1984). They examined several isolates of P. meadii and found that none of the isolates produced chlamydospores in culture. The lack of a method to produce chlamydospores preferably free of sporangia has hindered the progress of studies on the survival of chlamydospores. In order to assess the likelihood of P. meadii perennating in Sri Lanka as oospores or chlamydospores, the factors which might influence their formation and germination were investigated.

2. Literature review

Based on the ability to produce sex organs in single or paired cultures, species of Phytophthora are distinguished as homothallic or heterothallic (Ashby, 1922). In heterothallic species oospores are produced when paired with a complementary strain of the opposite mating type. Oospores are important propagules for long term survival and are major potential sources of variation in nature. Sexual reproduction allows genetic recombination potentially resulting in greater virulence and/or wider host range (Zentmyer & Erwin, 1970).

2.1 Factors influencing the production and germination of oospores.

2.1.1 Production of oospores.

(a) Temperature: Temperature appears to be one of the most important environmental factors affecting the formation of oospores in Phytophthora spp.. Turner (1961), Satchuthananthavale (1963) and Peries and Fernando (1966) found that oospores are formed by P. meadii at low temperatures (15° - 20°C) but not at higher temperatures. Brasier (1969) confirmed that the optimal temperature for the formation of oospores of P. palmivora (22°C) was lower than that required for its growth (26°C). Similar observations have been made for P. parasitica (Honour and Tsao, 1974) and P. cinnamomi (Zentmyer, et al, 1979). In contrast, the optimal temperatures for mycelial growth and oospore formation of P. hevea was found by Brasier (1969) to be similar.

(b) Light: Harnish (1965) studied oospore production in 10 species of Phytophthora including heterothallic as well as homothallic species and found that in most species white light at high intensity reduced or inhibited oospore formation. Abundant oospores were formed in continuous darkness in all species tested. In addition to darkness, red light favoured oospore formation in P. cactorum and P. capsici. Huguenin and Boccas (1971) found that exposure of P. palmivora to

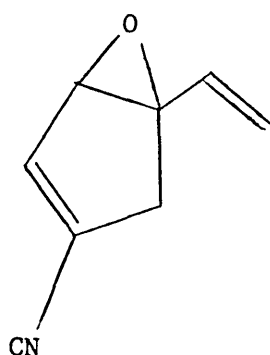
constant illumination at a range of light intensities was inhibitory to oosporegenesis, compared to that of alternate light and dark periods (12h : 12h). Huguenin and Jacques (1973) and Ribeiro et al. (1976) obtained stimulation of oospore production with P. cinnamomi even with daylight and blue light at low intensities.

There is also evidence that factors like temperature and light operate through hormones. Yu et al. (1981) found that hormone production was essential for oospore formation in P. parasitica A₁ compatibility type and that the production of hormone was optimal at 25°C and very poor at 30°C. They also found that when cultures of compatible strains were separated by a polycarbonate membrane, hormones were produced by P. colacasia A₂ and induced production of sexual organs by selfing provided the pairing was done in the dark.

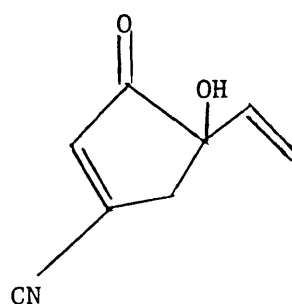
(c) Stimulation: Apart from induction by the opposite mating type other external factors have been found to induce oospore formation. The common soil inhabiting fungus Trichoderma viride Pers. ex S.F. Gray, was found to be effective in inducing sexual reproduction in many A₂ isolates of Phytophthora spp.. This effect was first suspected when an isolate of P. palmivora on carrot agar produced antheridia and oogonia after becoming contaminated with a Trichoderma sp., (Brasier, 1967).

The observation on P. palmivora has been followed by examination of isolates of different heterothallic species for response to T. viride. The stimulus has been effective in nearly all species tested so far, but seems to be confined to the A₂ compatibility type, though not all A₂ isolates have responded in the test conditions (Brasier, 1971; 1975). Fifty of the 62 A₂ isolates were tested by Brasier (1975), but none of the 34 A₁ isolates, responded to T. viride. All A₂ isolates of P. cinnamomi responded well; those of P. cambivora responded less well. Several strains of P. palmivora gave negative results. Exposure to the

Trichoderma sp., needed to be for 24h or less. Sex organs appeared in 2-4 days (Brasier, 1975). Pratt et al. (1972) found that oospores were produced in A₂ strains of P. cinnamomi in the presence of T. koningii. A series of experiments done later proved that volatile products of Trichoderma spp., were responsible for inducing oospore formation (Brasier, 1975; Pratt et al., 1972). Two iso-cyanides, homothallin I and II, have been identified as the active agent(s) in T. koningii vapours and their synthesis has been reported (Sakata and Rickards, 1980).



Homothallin I



Homothallin II

2.1.2 Germination of oospores

One of the major problems encountered in the study of oospores in species of Phytophthora has been the difficulty to germinate them consistently and in sufficient numbers to conduct genetic or physiological studies. As a result the progress of these studies have been hindered.

(a) Oospore maturity (age of oospores): Germination of oospores of some Phytophthora spp., have been found to increase initially with increasing age. Thus Shaw (1967) obtained no germination with 4-day-old oospores of P. cactorum but 67% germination with those which were

25-days-old. Similarly, Satour and Butler (1968) reported 2.6% and 20% germination of oospores of P. capsici from 14-day and 60-day cultures, respectively.

In contrast oospores of some Phytophthora spp., have the ability to germinate soon after they are formed provided they are given suitable conditions of light, temperature and nutrition as in P. fragariae (Duncan, 1977) and P. megasperma f.sp. medicaginis (Förster et al, 1981).

(b) Temperature: de Bary in 1881 first showed that chilling was an essential pre-requisite for germination of oospores of P. cactorum. Similar observations were made by Pethybridge (1914) with P. erythroseptica, Rosenbaum (1917) with P. cactorum; Hotson and Hartge (1923) with P. mexicana and Blackwell and Waterhouse (1931) with P. cactorum. However, germination of P. palmivora oospores was inhibited after they were chilled at 5° or 9°C (Kaosiri et al., 1980). It has also been observed that oospores require exposure to a lower or higher temperature than the ambient temperature prior to their germination. Pre-treatment of P. palmivora at 12° and 30°C for one week enhanced germination but it was suppressed when pre-treatment was done at 33°C (Kaosiri et al, 1980).

(c) Light: Several workers have demonstrated that light and light quality play an important role in germination of oospores. Shaw (1967) and Meyer (1975) reported that only a few oospores of P. cactorum germinated in the dark compared to those subjected to light. Exposure of P. cactorum to high light intensities for longer periods favoured oospore germination more than exposure to the same intensity for a shorter period followed by a longer dark period (Banihashemi and Mitchell, 1976) for P. cactorum. Banihashemi and Mitchell (1976 & 1980) found that maximum germination occurred under blue light for P. cactorum

and P. citricola. Huguenin and Boccas (1971) also reported similar behaviour in P. palmivora.

(d) Enzymes: Increase in germination of oospores after passing through a snail's gut was attributed to the enzymatic action of helicase or glusulase, a mixture of β -glucuronidase and aryl sulfatase (Gregg, 1957; Salvatore et al, 1973; Shaw, 1967). However, enzyme treatment does not appear to be effective in enhancing germination of oospores in all species of Phytophthora. Long et al. (1975) found that treatment of oospores of P. megasperma f.sp. glycinea was inhibited by treatment with 0.025% helicase while Förster et al., 1981 recorded 45% germination of P. megasperma f.sp. medicaginis after treatment of oospores with 5% (w/v) glusulase for 12h. More than 24h enzymatic treatment reduced germination. The enzymes protease, lipase and cellulase stimulated oospore germination.

2.2 Chlamydospores

Chlamydospores are asexual structures produced by some species of Phytophthoras and serve to maintain the population when the pathogen has almost ceased to grow due to adverse environmental conditions or tide over from one season to another. They are usually globose, thick or thin walled structures. There are several factors that influence their production.

2.2.1 Factors influencing production and germination of chlamydospores

(a) Medium: Chlamydospores can form readily in rich nutrient media eg. V8 juice supplemented with 0.1% calcium carbonate (Tsao, 1971; Kadooka and Ko, 1973; Chee, 1973) and also media depleted of nutrients (Tsao, 1971).

(b) Aeration: Lack of aeration appears to favour chlamydospore production by some species. Thus Tsao (1971) found that chlamydospores

of P. parasitica were produced in abundance when cultures were submerged about 5cm in a liquid medium. Huguenin (1974) obtained similar results with P. palmivora but Chee (1973) did not. He found that slightly wetting the mycelial mats induced abundant chlamydospores and some were formed even when mycelial mats were floated on the medium.

(c) Light: Although information on the influence of light on the production of chlamydospores for Phytophthora spp., is scanty, some work has been reported for a few species of Phytophthora. Zentmyer and Erwin (1970) reported that light did not influence production of chlamydospores of P. cinnamomi. Later work of Englander and Turbitt (1979) found no significant difference in chlamydospore production between cultures of P. cinnamomi irradiated with light of a spectral range between 470 - 725nm and cultures incubated in the dark. In P. lateralis chlamydospore production was suppressed in the light at intensities between 85-680 $\mu\text{W}/\text{cm}^2$ but was produced in the dark (Englander & Roth, 1980). However, P. palmivora behaved differently producing four times more ^{chlamydospores} in light than in dark (Zentmyer & Erwin, 1970).

(d) Temperature: Chlamydospores are produced over a wide range of temperatures. Chee (1973) reported that production of chlamydospores of P. palmivora could occur between 15° and 32°C with the optimum being 32°C. Huguenin & Boccas (1971) also reported a range of 10°-33°C for the same species with an optimum of 18°C. Wide ranges in temperature were also reported for P. lateralis (Englander & Roth, 1980), the values being 12°-26°C with an optimum of 24°-25°C and for P. parasitica (Tsao, 1971) with a range of 12°-30°C, the optimum being 15°-18°C. Although the range for most species appear to be similar, the optimum temperature for chlamydospore production seem to vary with the species.

2.2.2 Germination of chlamydospores

Very little information is available on the germination of

chlamydospores of Phytophthora spp., particularly those affecting rubber and cocoa. Temperature does not appear to be a critical factor in chlamydospore germination. Chee (1973) reported that chlamydospores of P. palmivora from rubber germinated over a wide range of temperatures between 15^o-32^oC and that the optimum was between 26^o-29^oC. For P. cinnamomi germination was observed from 9^o-36^oC with the optimum being 20^o-30^oC (Mircetich et al., 1968). Age of the chlamydospore seemed important for P. palmivora, where highest germination was recorded for one week old chlamydospores and none was found to germinate when they were 3 months old (Chee, 1973). However, for P. cinnamomi (Mircetich et al., 1968) and P. parasitica (Holdaway and Tsao, 1972) age did not have any influence. Chlamydospores of some species such as P. palmivora do not require exogenous nutrients for their germination and are thus able to germinate on water agar (Chee, 1973, Kadooka & Ko, 1973). Water agar when supplemented with glucose and asparagine did not show any improvement in germination when compared to that of water agar alone (Huguenin, 1974). He also found that amino acids inhibited germination of chlamydospores in P. palmivora. Mircetich et al., 1968 showed that low concentrations of sugars (2.5mM) did not induce chlamydospore germination in P. cinnamomi but it was improved when sugars were present in high (5g/L sucrose) concentrations. All 11 amino acids tested stimulated chlamydospore germination in P. cinnamomi (Mircetich et al., 1968; Tsao, 1969; Tsao and Bricker, 1968). Similar results were obtained with P. parasitica (Tsao & Bricker, 1968). However, chlamydospore germination in P. palmivora was inhibited by amino acids (Huguenin, 1974). It has also been reported that chlamydospores of P. cinnamomi germinated by producing many germ tubes when placed in soils amended with glucose (0.05M) or asparagine (0.0125M) or combinations of glucose and asparagine or glucose and NaNO₃, (Mircetich & Zentmyer,

1969). In non-amended natural soils most of the chlamydospores that germinated formed a germ tube terminating in a germ sporangium. For P. parasitica the addition of glucose (0.4%); asparagine (0.4%) or NH_4NO_3 (0.4%) to soil did not enhance germination of chlamydospores.

Thus species of Phytophthora respond differently to the presence of Carbon and nitrogen in the soil and in artificial media.

3. Materials and Methods

The same basic methods described in Chapter I were used. Particular methods which are not included there, are described with the individual experiment.

4. Results

4.1 Factors influencing production of oospores

4.1.1 Light/dark

Table 25 shows the number of oospores formed at different light regimes at 20°C, when two compatible isolates of P. meadii (Isolate no. 126 x Isolate no. 22L) were paired on V8 agar. Oospore production was significantly higher in total darkness than in alternate light or continuous light.

4.1.2 Temperature

(a) Pairings of P. meadii isolates : Lower temperatures were more conducive for oospore production than high temperatures, for pairings of P. meadii isolates. The highest oospore number was recorded between 18°C-22°C with the maximum number being formed at 20°C. Numbers of oospore reduced considerably beyond 22°C but the number produced at 30°C was no less than at 25°C (Table 26).

(b) Production of oospores in P. citricola (Homothallic isolate) : Maximum production of oospores in both the light and dark regimes occurred at 20°C though the numbers formed were not much higher than at 25°C. At 30°C there was still a considerable number of oogonia but few

Table 25. Production of oospores in pairings of P. meadii isolates at different light regimes at 20°C.

Treatment	^a Oospores/mm ²			
	Replicate 1	Replicate 2	Replicate 3	Mean
Continuous light	8.7	8.2	8.6	8.49
Continuous dark	85.8	85.2	84.9	85.30
Light/dark	25.8	19.7	18.7	21.40

^a Each figure is a mean of 50 observations S.E. 1.7

	D.f.	SS	MS	F
Treatment	2	10151.28	5075.64	1068.74***
Error	6	28.50	4.75	
Total	8	10179.77		

Table 26. Production of oospores in pairings of P. meadii isolates at different temperatures

Temperature°C	^a Mean oospores/mm ²
30	35.1
28	33.5
25	35.3
22	72.7
20	77.4
18	72.9
	S.E. 2.84

	D.f.	SS	MS	F
Treatment	5	19868.6	3973.7	359.80***
Error	84	927.7	11.0	
Total	89	20796.3		

^a Each figure is a mean of 15 observations.

at 15°C. At each temperature studied, more oospores formed in the dark (Table 27). In the culture plates, oospores were observed, in abundance, in 48h at 20°C in both light and the dark. At temperatures of 25° and 30°C these were produced in 48h, only in total darkness but in the light, a longer period of 72h was required. At 15°C, in both the light and dark regimes, oospores were formed only after 96h incubation period.

4.1.3 Stimulation by Trichoderma species.

Several isolates of P. meadii (Isolate no. 126, 22L, 9P, 362 and 26B) were grown on 10ml V8 agar. Trichoderma spp., T. lignorum, T. harzianum and T. koningii which are the common Trichodermas present in rubber soils (identified by the CMI) were inoculated on to separate dishes containing 10ml V8 agar. On the 6th day, when the Phytophthora colony had reached a diameter of 6-8cm, the lids of both dishes were removed, the Phytophthora culture inverted over the Trichoderma culture and the dishes sealed together with tape. All treatments were replicated five times. Phytophthora cultures not exposed to Trichoderma served as the controls.

The cultures were examined after 10 days. Oospores were observed only when cultures of the A₂ compatibility type of P. meadii (isolate no. 126) were combined with T. koningii. Other Trichoderma species did not stimulate oospore production either in the A₁ or A₂ compatibility types. Counts of oospores on 50 agar plugs showed that isolate 126 produced a mean of 15 oospore/mm² when combined with T. koningii and 47 oospores/mm² when paired with isolate 9P, a compatible P. meadii isolate.

4.2 Factors influencing production of chlamydospores.

4.2.1 Temperature/light

Plugs, 6mm diameter from the edge of a 4-day old culture of P.

Table 27. Production of oospores in P. citricola at different temperatures in continuous light and total darkness.

Temp ^o C	Light/ Dark	No. oospores/mm ² a											
		Replicates											
		1	2	3	4	5	6	7	8	9	10	Total	Mean
15	L	8	6	14	7	11	10	6	2	0	4	68	6.8
	D	7	9	12	10	9	14	10	5	3	9	88	8.8
20	L	65	73	81	62	75	86	69	73	69	70	723	72.3
	D	80	82	79	92	112	82	76	85	102	76	866	86.6
25	L	56	61	39	86	26	80	59	72	83	61	637	63.7
	D	86	73	51	92	101	56	83	61	92	96	791	79.1
30	L	43	36	62	59	47	36	51	69	46	39	488	48.8
	D	59	84	69	41	56	74	67	85	96	49	680	68.0

^a Each figure is a mean of 5 observations.

Source	D.F.	S.S.	M.S.	F
Temp	3	63362	21120	6.95 [*]
Light	1	2797	2797	19.02 ^{***}
Int.	3	785	262	1.78
Error	72	10614	147	
Total	79	77558		

meadii (isolate no. 27L) grown on V8 juice agar were placed individually in 350ml medical flats containing 25ml of clarified V8 juice. After incubation at 25°C in the dark for 24h, each bottle was shaken vigorously by hand to fragment the hyphae. Care was taken to re-suspend the fragments adhering to the wall of the bottle, by gently rotating the bottle. The bottles were then incubated horizontally as stationery cultures at 25°C for 1 week by which time mycelium, free of any spore forms, had covered most of the surface of the medium. One hundred ml. of sterile de-ionized water was added to each bottle and these were incubated vertically at 10°, 18°, 20°, 25° and 30°C. One set was left under continuous light and the other under total darkness. Six weeks after incubation, mycelium was fragmented using a Sorvall Omni mixer and the number of chlamydospores were assessed in 10 microscopic fields per each replicate.

Production of chlamydospores was observed in some selected isolates of P. meadii (Nos. 27L, 16P, 9P). Other isolates did not produce any chlamydospores (Nos. 4P, 22L, 23P) or produced only a few (126, 14P). However, a large number of chlamydospores were formed in isolate 27L at 20°C. At 15°, 18° and 20°C chlamydospores were produced free of sporangia, although occasionally a few sporangia were seen. The optimum temperature for chlamydospore production in isolate 27L was 20°C. Darkness favoured chlamydospore production while continuous light was detrimental to it (Table 28). At high temperatures (25° and 28°C) a large number of sporangia were observed. Chlamydospores produced at 18°, 20°, 25° and 28°C, in the dark were of similar size (31µm - 34µm) but those formed at 10°C were much smaller (c. 7µm).

A separate experiment was done to find out whether a medium containing rubber petiole extract could be used to produce chlamydospores of P. meadii. The medium was prepared as given in

Table 28. Production of chlamydo spores in P. meadii (isolate no. 27L) on clarified V8 broth at different temperatures and light regimes.

Temp ^o C		^a No. chlamydo spores/5 fields						Total	Mean
		R ₁	R ₂	R ₃	R ₄	R ₅	R ₆		
10	L	20	7	6	17	15	8	73	12.2
	D	232	256	251	256	173	210	1378	229.7
18	L	25	15	9	29	19	13	110	18.3
	D	189	245	193	245	232	282	1386	231.0
20	L	61	38	22	18	36	30	205	34.2
	D	236	286	295	286	250	244	1597	266.2
25	L	0	0	0	0	0	0	0	0
	D	10	14	12	14	14	8	72	12.0
28	L	0	0	0	0	0	0	0	0
	D	4	2	5	2	2	2	17	2.8

^a No. chlamydo spores in 5 microscopic fields (3.8mm²) for each replicate.

L - continuous light condition

D - continuous dark condition

Appendix I. The experimental procedure was as given in Chapter 2, 4.2.1. except that the cultures were incubated in total darkness as information from the previous experiment showed that more chlamydospores are produced in total darkness. Only isolate no. 27L was used.

The optimum temperature for chlamydospore production was found to be 20°C in petiole extract too. At high temperatures (25° and 28°C) a large number of sporangia were observed as in the previous experiment. At 15°, 18° and 20°C chlamydospores were produced free of sporangia. The total number of chlamydospores produced in petiole extract was however, less compared to that on V8 juice (Table 29).

4.2.2 pH

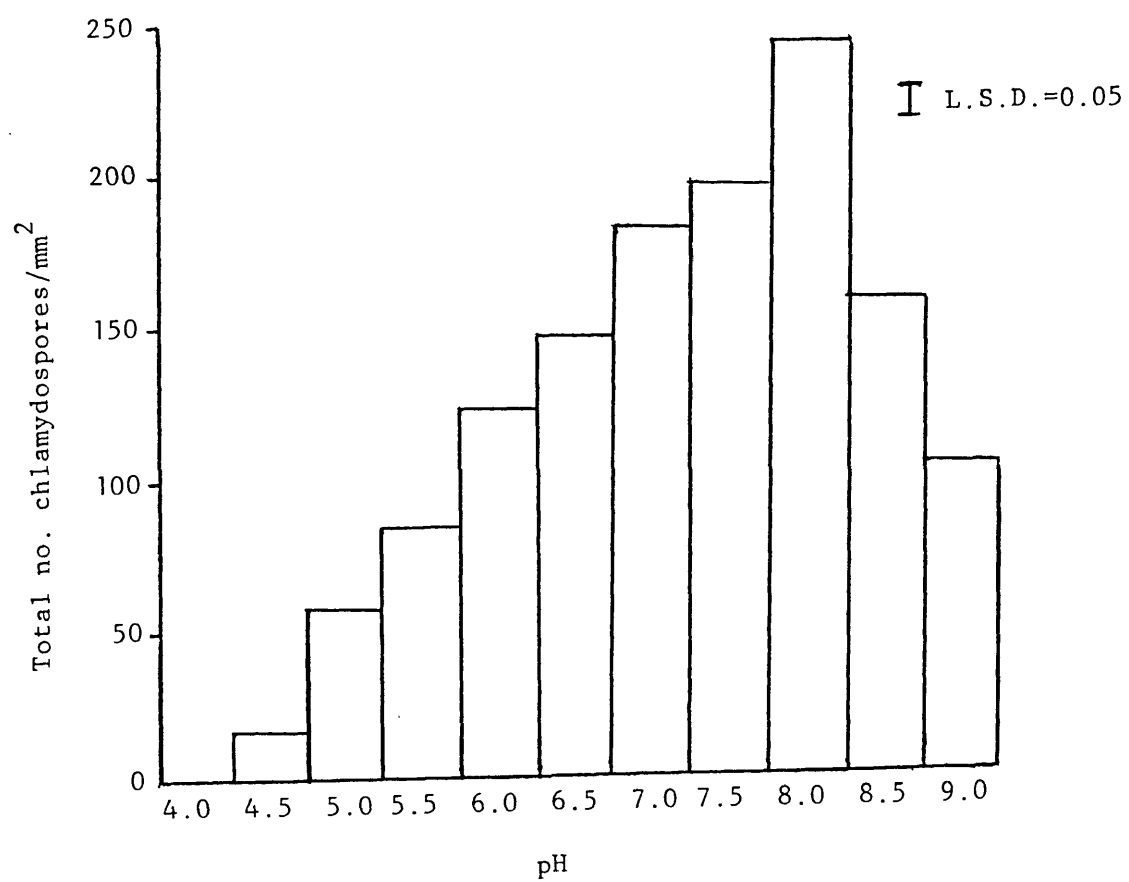
This experiment was carried out in a manner similar to that described in 4.2.1. The pH of clarified V8 juice was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 using 0.1N NaOH or 0.1N HCl. Isolates were incubated at 20°C in total darkness and observations were made 6 weeks after incubation as described in 4.2.2.

Figure 18 shows that pH 8.0 was optimum for chlamydospore production but that P. meadii has the ability to form chlamydospores over a wide range of pH values. Relatively a few chlamydospores were formed below pH 5.0 but their production increased progressively upto pH 8.0 when the maximum number was recorded. At pH values above 8.0, the number of chlamydospores formed, decreased.

4.3 Effect of different substrates on germination of oospores.

Oospores of P. meadii (intra-specific pairing of isolates 126 and 9P) and that of the homothallic isolate P. citricola were produced on LBA at 20°C. Oospores were obtained by macerating the culture, in a Waring blender, with sterile distilled water for 3 min. The suspension containing oospores was filtered through sterile muslin cloth to obtain oospores free of mycelial fragments. One ml. of the suspension

Fig. 18. Effect of pH on production of chlamydospores in P. meadii
(isolate no. 27L) at 20°C in total darkness.



containing 7-day old oospores was placed in each of the 28 Universal containers and a similar set was prepared with 30-day old oospores. Half of the containers in each set were kept at 10°C and the remainder at 30°C for one week. One ml. of one of the following materials was then added to 4 containers in each set. These were: 0.5M glucose, 0.01M L-Asparagin, 0.01M amylase, 0.01M diastase, 0.01M cellulase, 2% soil extract and de-ionized water. Within each of these treatments, one container was incubated in total darkness and one in continuous light, both at 25°C. A similar experiment was carried out with seven and 30-day old oospores of P. citricola.

Pre-treated oospores both young and mature, in the intra-specific pairing did not germinate under the conditions tested. No oospores germinated in de-ionized water. In P. citricola germination was observed after low and high temperature pre-treatment, in the presence of de-ionized water, enzymes and nutrients. No increased germination was observed due to pre-treatment, nutrients and enzymes. It was also observed that some of the 7-day old oospores germinated without any pre-treatment. The percentage oospores germinated was low (4%-10%) under all conditions tested and therefore, a quantitative study was not done. All germinated oogonia produced a single germ tube mostly from the antheridial wall (Fig. 19) or a very few from oospore wall (Fig. 20).

4.4 Germination of chlamydospores.

Six week old chlamydospores of P. meadii isolate 27L were produced as in 4.2.1 and macerated in a Waring blender with 100 ml de-ionized water approximately for 3 min. until they were separated from the mycelium. The suspension was filtered through a sterilized muslin cloth. One ml. of chlamydospore suspension was placed in Universal containers. An equal volume of 0.5M glucose, 0.01M L-asparagine, 0.01M

amylase, 0.01M diastase, 0.01M cellulase and 2% soil extract was added to each container. Bottles were incubated at 25°C in alternate light (12h/12h) and dark for 24h. Drops of suspension were examined for germinated chlamydospores.

Germination of chlamydospores in different substrates is given in Table 30. In the presence of glucose, L-asparagine and enzymes chlamydospores germinated well (89%-99%) producing germ tubes which ramified extensively (Fig. 21) whereas in the presence of 2% soil extract most chlamydospores germinated producing germ tubes which terminated in a germ sporangium (Fig. 22). No chlamydospores germinated in de-ionized water.

4.5 Effect of some selected isolates of P. meadii on rubber petioles.

Twenty three isolates were selected from the P. meadii group for this study. This consisted of 10 each from petiole and leaf isolates and three from bark/shoot isolates. Tender petioles of rubber (clone PB86) were inoculated with the above isolates in the same manner as in Chapter 1.2.9., to determine whether the isolates differ in their ability to induce lesions.

It was observed that the lesion size varied with each isolate. Some isolates produced longer lesions while some produced smaller lesions. Petiole isolates as a group did not have any particular advantage on petioles compared with leaf isolates. Although only a few bark/shoot isolates were tested, they appeared to give long lesions than petiole or leaf isolates. The lesion size was not linked with the growth rate on agar (Table 31).

Table 29. Production of chlamydospores in P. meadii (isolate no. 27L)
on petiole extract at different temperatures in total darkness

Temp ^o C	^a No. chlamydospores/5 fields						Total	Mean
	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆		
10	41	32	50	37	37	50	274	41.2
18	77	94	52	111	74	44	452	75.3
20	129	119	123	160	111	163	805	134.2
25	25	50	8	13	13	19	128	21.3
28	0	0	0	0	2	3	5	0.8

^a No. chlamydospores in 5 microscopic fields (3.8mm²)
for each replicate.

Table 30. Germination of chlamydospores on different substrates after
24h. incubation at 25^oC.

Treatment	germination ^a
0.05M glucose	97.7 ± 5.71
0.01M L-asparagine	88.8 ± 3.54
0.01M amylase	98.0 ± 4.89
0.01M diastase	99.0 ± 2.44
0.01M cellulase	98.0 ± 3.16
2% soil extract	35.7 ± 7.85
De-ionized water	0

^a Each reading is a mean of 6 observations.

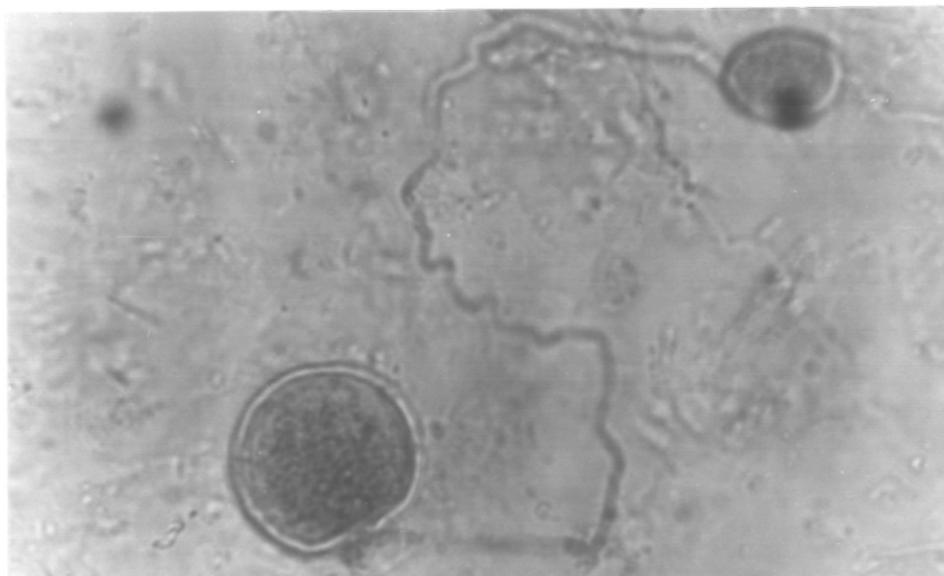


Fig. 19 A germinated oogonium with germ tube arising from the wall of the antheridium.



Fig. 20. A germinated oospore (*P. citricola*) with germ tube arising from the wall of the oospore.

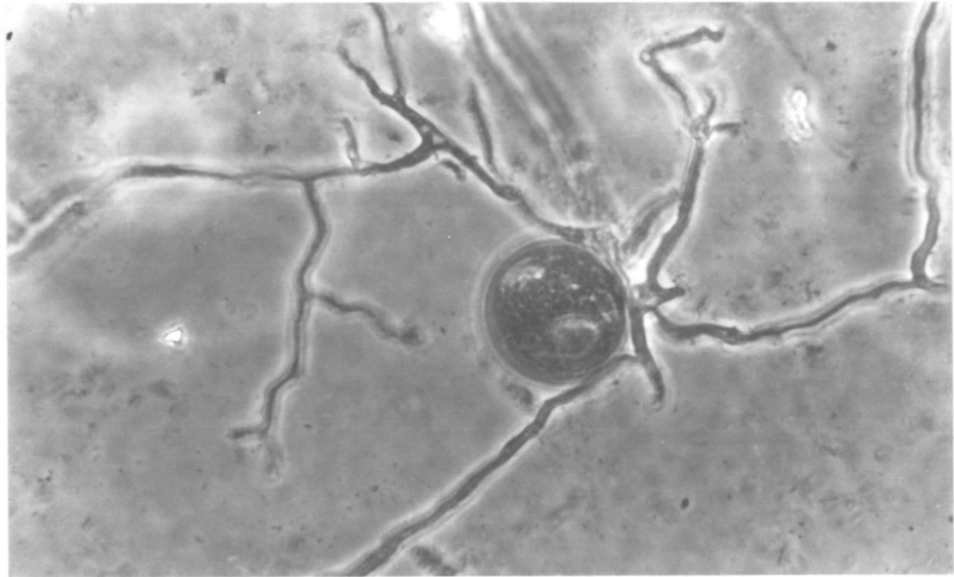


Fig. 21. Germinated chlamydospore in 0.5M glucose. Note the extensively branched germ tubes.

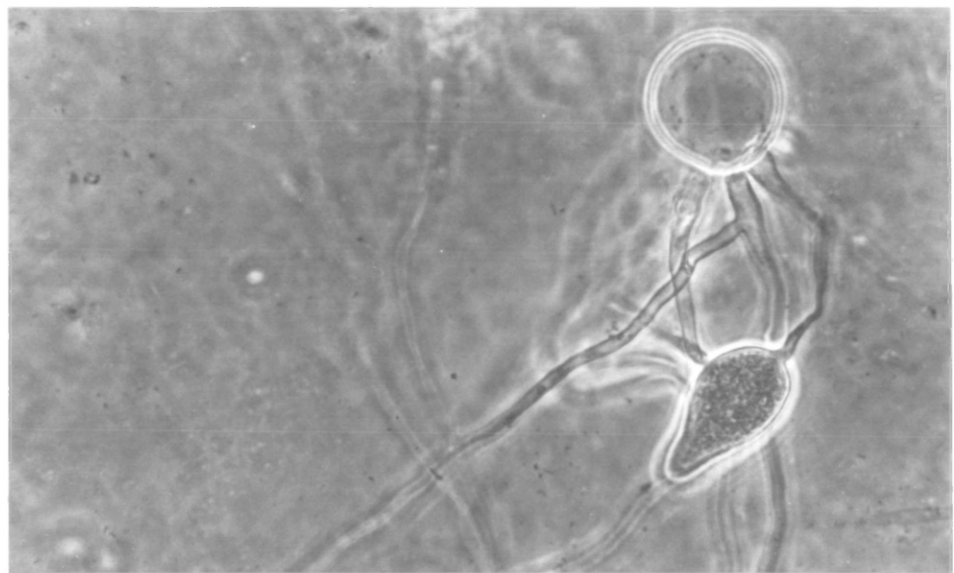


Fig. 22. Germinated chlamydospore in 2% soil extract. Note germ tube ending in a sporangium.

Table 31. Lesion length on rubber petioles inoculated by P. meadii isolates and the mean growth of isolates.

Isolate no.	Source	Mean lesion length (mm)	Mean growth of isolates between 1-4 day (mm)
4P	Petiole	13.0	35
11L	Leaf	13.8	37
4L	Leaf	16.6	36
2P	Petiole	16.8	42
1P	Petiole	17.2	38
22L	Leaf	17.8	34
6P	Petiole	18.2	34
27L	Leaf	18.6	33
5P	Petiole	18.8	38
21L	Leaf	19.0	34
25P	Petiole	19.2	31
7P	Petiole	20.6	43
28P	Petiole	20.6	39
26L	Leaf	21.0	35
24P	Petiole	21.4	35
18L	Leaf	23.2	37
3L	Leaf	24.0	39
16L	Leaf	25.0	40
14P	Leaf	25.2	38
5L	Leaf	26.4	43
12B	Bark	27.0	42
26S	Shoot	28.0	38
29B	Bark	28.2	29

C.F. = 50003.51 ^a Each figure is a mean of 3 replicates

Source	Df	SS	MS	F
Treatment	22	2083.287	94.6949	12.28***
Error	92	709.200	7.7086	
Total	114	2792.487		

DISCUSSION

One of the primary objectives of the experiments described in this chapter was to determine some of the conditions under which oospores and chlamydospores of P. meadii formed and germinated so that large numbers of these could be obtained for experiments especially on survival.

Incubation of paired cultures in the dark at temperatures between 18° and 22°C proved most favourable for the formation_{of} oogonia^{and antheridia}. At higher temperatures (25°-30°C) the numbers of oogonia were considerably reduced (to about half of those at 22°C) and many sporangia formed. The rapid decline in production of oogonia between 22° and 25°C was particularly striking and suggests that some critical stage in formation of oogonia is particularly sensitive to temperature. In contrast to the results of both Turner (1961) and Satchuthananthavale (1963), who obtained no oogonia above 28°C, moderate numbers were formed at 30°C in this study. Oogonia formed abundantly in the dark, with light apparently inhibiting oogonia production.

Similarly, chlamydospores formed most abundantly in the dark and at temperatures below 20°C, even as low as 10°C. It might be supposed that temperatures generally in the rubber-growing areas of Sri Lanka are too high to favour either the production of oogonia or chlamydospores. However, although the daytime temperatures are usually between 25° and 28°C, at night they fall to 20-25°C especially at altitudes of 100-300M. In this range, oogonia at least could form.

Of the Trichoderma spp. tested, only T. koningii induced oogonia in P. meadii and, as others have found with different species, only the A₂ compatibility type responded in this way. Pratt et al. (1972) also found that T. koningii stimulated oogonia production by P. cinnamomi but with this species T. viride has also been shown to be effective (Brasier, 1971, 1975). However, although T. koningii is commonly

present in soils of rubber plantations in Sri Lanka and on root surface, the chances of it stimulating oogonia production in P. meadii appear remote because the A_1 type predominates, not the A_2 type. It seems unlikely, therefore, that formation of oogonia in this way plays any significant role in the epidemiology of Phytophthora disease on rubber.

There have been relatively few studies on the production of oogonia and chlamydospores of P. meadii in-vitro, so the results with V_8 $CaCO_3$ broth are of particular interest. The method could be used to produce large numbers of chlamydospores for experiments, especially those on survival. Although chlamydospores do not normally form on V_8 agar, submerging the culture in liquid medium stimulated their production suggesting that reduced aeration may be an essential feature. Although under similar conditions fewer chlamydospores formed on petiole extract this medium could be substituted for V_8 $CaCO_3$ broth.

Also, pH appeared less critical to the formation of chlamydospores, substantial numbers have been formed in the range 4.5 to 9.0. Rubber soils generally have a pH of 5.0-5.5 and the pH of rubber tissue varies between 6.0 and 6.5. It therefore is unlikely that pH itself would limit chlamydospore production.

Even if oospores and chlamydospores of P. meadii form, there remains the uncertainty of their role in the epidemiology of the diseases because, in-vitro, it is often difficult to get them to germinate. This applies particularly to oogonia. In the present study, similar techniques to those stimulating germination in other species such as P. cactorum, P. palmivora and P. capsici, failed with P. meadii. Whether the techniques themselves were unsuitable or whether oospores of P. meadii require some period of maturation before they are capable of germinating requires further investigation. In contrast, chlamydospores germinated well if provided with exogenous nutrients and this makes it

likely that they would do so in the rhizosphere of rubber. So far, root infection by P. meadii has not been observed in Sri Lanka but this does not preclude the possibility that germination occurs near roots and zoospores are subsequently dispersed by rain splash as they are with other species, for example P. megakarya on cocoa.

P. citricola is newly reported as a species occurring on rubber in Sri Lanka so any data on the ability of this isolate to produce oogonia and for these to germinate are of special interest. Oogonia were produced in abundance at 20°, 25° and 30°C both in the light and dark and so P. citricola appears well adapted to produce them under field conditions. However, its pathogenicity to rubber is low and therefore it is unlikely to cause severe damage even if it became widespread. A few oospores germinated without special treatment but the germ tubes in most cases appeared to originate from the antheridial wall and a very few through the oospore wall. The ability of this isolate to germinate effectively and its potential to give rise to variants remain in some doubt.

CHAPTER 3

SURVIVAL OF PHYTOPHTHORA MEADII

1. Introduction

Different species of Phytophthora are known to survive for long periods as dormant or resting propagules such as chlamydospores and oospores. It is possible that some species persist for short period as mycelial fragments, sporangia or zoospores but information is generally lacking as it is also for the survival generally of Phytophthora spp. attacking rubber.

Studies on survival rely on suitable techniques for isolating Phytophthora : Isolation from fresh lesions on plants is readily achieved by plating tissues from the margins of the lesion onto suitable agar. Difficulties arise when the tissue is badly decayed, old or dried and when isolation of Phytophthora from soil is attempted. In these instances techniques such as baiting and the use of selective media are invaluable.

The selective isolation of Phytophthora spp. from soil depends on the proper choice and use of plant parts as baits. The many individual techniques can be categorized into three groups.

(a) Inserting soil or infected tissue into a hole made on a fruit from a susceptible host to which the fungus is pathogenic e.g. use of apple (Cambell, 1949) and avocado (Zentmyer et al, 1960) for P. cinnamomi; cocoa pod for P. palmivora (Chee & Foong, 1968).

(b) The use of seeds, seedlings and rooted cuttings in soil, in pots subjected to heavy watering or in the field exposed to rain. e.g. Cantaloupe seed for P. drechsleri (Alavi & Strange, 1979); avocado seedlings for P. cinnamomi (Zentmyer & Ohr, 1978); strawberry rooted

runner for P. fragariae (Duncan, 1976).

(c) Floating or partially immersing host tissue in a mixture of soil and water e.g. Eucalyptus cotyledons for P. cinnamomi (Marks & Kassaby, 1974); cocoa pod tissue for P. palmivora (Newhook & Jackson, 1977). Among susceptible hosts or plant parts, some have been found to be more sensitive as baits than others for a given Phytophthora sp.. For isolating P. cinnamomi from soils, leaf discs of Rhododendron spp. were more successful than blue lupin radicles (Shew et al., 1979); lupin radicles were excellent for isolating P. megasperma but unsuitable for isolating P. cactorum (Dance et al. 1975).

Use of selective media for detection and isolation of fungi was initiated in the early 1960s. Phytophthora was one of the first genera of plant pathogenic fungi for which selective media were devised (Tsao, 1970). Polyene antibiotics are known to be strongly antifungal except to a small number of fungi, including those in the Pythiaceae. Eckert & Tsao (1960, 1962) were among the first to report the non-inhibitory nature of nystatin and pimaracin to Phytophthora and Pythium spp. Each of these two antibiotics have since been widely used in most selective media. Pimaracin is more potent and has a wider spectrum than nystatin and is generally more effective of the two.

Use of P10VP medium containing pimaracin, vancomycin and pentachloro-nitro-benzene (PCNB) has facilitated isolation of various Phytophthora spp. from freshly infected plant tissues and heavily infested soils. Isolations can be made by placing a dry or wet soil mass or by sprinkling small crumbs of soil, on the medium surface (direct inoculation), or by pouring cooled molten agar over a small quantity of soil suspension. Recovery of Phytophthora from badly deteriorated plant tissue laden with secondary micro-organisms is more difficult. However, P10VP medium, allowed isolation of Phytophthora spp from small pieces of

old dried badly decayed, infected rubber tissues with or without prior sterilization (Tsao et al, 1976).

Specific selective media for different Phytophthora spp. have been reported by many workers for example, corn meal agar with pimaracin, polymixin, penicillin and PCNB to isolate P. megasperma var. sojae from soil (Haas, 1964); potato dextrose agar with pimaracin, penicillin and neomycin for isolating P. citricola from walnut roots (Green & Pratt, 1970); and Pimaracin, penicillin and PCNB to isolate P. palmivora from leaf debris in cocoa plantations (Newhook and Jackson, 1977).

Despite the development of selective media and other techniques, there is relatively little information on how Phytophthora species survive from one season to the next. The data available suggest that generally mycelium, zoospores and sporangia survive for shorter periods than chlamydospores or oospores though there is a great deal of variation between species in the capacity of each propagule to survive and sometimes in reports of different workers using the same species. Thus P. cactorum is reported to survive in soil as mycelium for 3 days (Sneh & McIntosh, 1974) or 14 days (Gisi & Meyer, 1973), as zoospores and sporangia for 7-35 days (Sneh & McIntosh, 1974), as chlamydospores for more than 105 days (Gisi & Meyer, 1973) and as oospores for more than one year (Legge, 1953). Similar reports for the survival of P. cinnamomi are one day (Huwang & Ko, 1978) or 60 days (Malajczak & Theodorou, 1979) for mycelium and 84 days (Huwang & Ko, 1978) or a year (Weste & Vithanage, 1979) for chlamydospores.

Data for the survival of Phytophthora species associated with rubber are particularly sparse. Turner (1965) reported that P. palmivora from cocoa survived as sporangia in soil for 10 to 108 days. Chee (1973), particularly, examined the survival of P. palmivora as chlamydospores and mycelium. He found that chlamydospores were killed by drying for 4.5h.

Also, no chlamydospores could be detected in soil one week after it had been inoculated with a chlamydospore suspension. When petioles of rubber artificially inoculated with P. palmivora were placed in soil, many chlamydospores ($25-30/\text{mm}^2$) were found in the tissues after 4-5 weeks but by 6 weeks production of chlamydospores ceased and only empty chlamydospores were seen after 7 weeks. In contrast, soil artificially inoculated with mycelium yielded P. palmivora in isolations upto 32 weeks. He concluded, therefore, that chlamydospores were not important survival structures for P. palmivora and that this fungus persisted in soil mainly as hyphae. The experiments described in the chapter investigated three aspects of the survival of P. meadii.

- (1) The survival of mycelium, sporangia and chlamydospores in soil in the absence of the host.
- (2) The recovery of propagules from soil in a rubber plantation where the disease occurs each year, using a selective medium and baiting with cocoa pods.
- (3) The survival as chlamydospores or other forms in infected petioles of rubber.

2. Materials and methods

2.1 Survival of mycelium in soil

Plugs 6mm diameter from a 4-day old colony of P. meadii grown on LBA were transferred to 25 ml of petiole extract medium containing 20g of sucrose /L, (see appendix I) in 350 ml medical flats. These were laid flat and incubated in darkness for 48h at 25°C . The bottles were then shaken vigorously to break up the mycelia which had formed. One ml of mycelial suspension was added to 15 ml of petiole extract in 9 cm petri dishes. These were placed at 25°C in the dark for 72h. Under these conditions, only mycelia are formed in culture. Each mat, approximately 1.5 cm diameter, was floated onto a 2.5 cm square piece of nylon-mesh

(pore size 25 μm). Three nylon-mesh squares were buried approximately 1 cm deep in a petri dish containing 40g sieved (200 mesh) soil adjusted to 50% moisture holding capacity (MHC). Such plates were prepared and these were then divided into two sets. Those in set 1 were securely sealed with tape to prevent evaporation of soil moisture. Those in set 2 were not sealed, thus the soil in each plate was allowed to dry but every 14 days was rewetted by adding 5 ml of water per plate. The plates were left at room temperature (R.T.) $28^{\circ}\text{C} \pm 2$. Three nylon-mesh pieces from a single plate were examined weekly, from each set. On each piece 20 randomly selected squares were examined ($\times 400$). Mycelial lysis (visually as 0, 25, 50, 75 and 100%) and the number of chlamydospores and sporangia formed were recorded.

Each nylon-mesh piece was then washed thoroughly in sterile distilled water and cut up into small pieces, approximately 3mm^2 . Thirty of these pieces were plated on P10VP medium and viability of the mycelium determined from growth after incubation for 4-5 days at 20°C in the dark.

In a subsidiary experiment, squares of nylon-mesh with mycelium (prepared as above) were placed either in plates of sieved soil at 50% MHC which were sealed or in plates of air-dried soil (3-5% moisture) which were not sealed. Both sets were kept at room temperature and one piece of nylon-mesh from each set was examined daily for 6 days. In this instance, lysis and production of sporangia and chlamydospores only were recorded from an examination of 10 squares selected at random on each piece of nylon mesh.

2.2 Survival of sporangia in soil.

P. meadii was grown in 500 ml Erlenmeyer flasks containing 100 ml petiole extract medium for 7 days at 25°C in the light and the sporangia dislodged using a wrist-action shaker. The resulting suspension was filtered through muslin to remove mycelial fragments, centrifuged at

1000g. for 1 min and the concentration adjusted to 10^4 sporangia/ml. Drops (0.1ml) of sporangial suspension were placed on squares (1.5 cm^2) of nylon-mesh and these were buried in soil at 50% MHC in petri dishes in two sets as described in 2.1. Three mesh pieces were examined daily in the first week and weekly thereafter to determine the state of the sporangia. Each week the viability of P. meadii in the soil was tested by isolations on P10VP medium as in 2.1 above.

2.3 Survival of chlamydospores in soil

P. meadii was grown on petiole extract in the dark at 20°C for 4 months (see Chapter 2, Section 4.2.1). The fungal mats from 10 cultures were macerated in a Waring blender for 3 min with 100 ml deionized water. The suspension was filtered through sterile muslin to remove mycelial fragments and centrifuged at 1000g for 1 min to concentrate the chlamydospores. The final suspension was then adjusted to 10^3 chlamydospores/ml. Survival was examined by placing drops (0.1ml) on squares of nylon-mesh and burying these in soil as described for sporangia (2.2 above).

2.4 Detection of the fungus in soil by plating on selective medium and by baiting with cocoa pods.

Three sites in Dartonfield Estate where the disease is known to occur every year were selected in April 1985. This is generally the period of the year when the disease appears on immature rubber pods, although none were observed at the start of the experiment. The ground cover was removed from an area of 30 cm^2 at each site and soil collected to a depth of 3cm. The samples from the three sites were pooled and mixed thoroughly. Ten subsamples c.0.05g of the pooled sample were air dried for 1h on sterile blotting paper. Each subsample was spread out in a sterile petri dish, 15ml of cooled ($43^\circ\text{--}45^\circ\text{C}$) P10VP medium was added and the plate rotated to distribute the soil particles evenly. Plates

were incubated at 20°C in the dark and colonies observed from the third day. The number of plates with Phytophthora colonies was recorded.

Baiting with cocoa pods was carried out as follows:

Mature pods of Amelonado cocoa were surface sterilized with 70% alcohol. Two holes were made with a 1 cm diameter cork borer approximately 1 cm deep on either side of each pod midway between stalk end and tip. Approximately, 2g soil were placed in each hole and a few drops of sterile water added to moisten the soil. Each hole was covered with sellotape and the pods were kept in trays lined with moist blotting paper. When characteristic lesions appeared the pathogen was isolated by removing a small piece of tissue aseptically from beneath the lesion surface and placing it on lima bean agar. Eight cocoa pods were used per pooled sample.

2.5 Survival in infected rubber petioles in soil

2.5.1 Laboratory tests

Young petioles from clone PB86 were inoculated with zoospores (10^4 /ml) of P. meadii (isolate 362, A₁ compatibility type) and incubated in trays, lined with wet blotting paper, at R.T. When greyish-black lesions appeared (3-4 days) each petiole was cut into three lengths (c. 7 cm). These were placed in petri dishes and covered with sieved (200 mesh) soil, adjusted to 50% MHC collected from an area free of Phytophthora disease.

The plates were divided into two sets as in 2.1 above - one set sealed to prevent loss of moisture and the other not but with the soil re-wetted every fourteenth day. Each set was then subdivided into three subsets, one of which was incubated at 20°, 25° or 28°C. Uninfected lengths of petiole, similarly treated, served as controls.

Six lengths of petiole were removed from each treatment for examination at 2-week intervals to 40 weeks. Three lengths were

macerated and the macerate examined unstained for mycelium, sporangia, chlamydospores and oospores or after clearing in chloral hydrate and staining with cotton blue in lactophenol. The propagules in 10 fields (x 400) were recorded.

The other three lengths of petiole were washed thoroughly in tap water, then in several changes of sterile distilled water and finally in 5 ml sterile distilled water. Initially, P. meadii was recovered from the macerate by dipping the ends of young rubber petioles in it for 24h, incubating these until lesions developed and isolating from the lesions by plating pieces on P10VP medium. Subsequently, portions (0.05 ml) of the macerate were placed in wells (6 mm diameter) cut in plates of P10VP medium which were then incubated at 25°C in the dark. Twenty isolations of this type for each treatment were done at 2-week intervals to 30 weeks.

2.5.2 Field tests

Infected petioles were obtained and cut into lengths of approximately 7 cm as in (2.5.1) above. Twelve lengths were placed in each of 40 nylon net bags (8 x 6 cm) and these were buried 3 cm below the soil surface in a plantation of mature rubber trees where there was no disease. Uninfected lengths of petiole in nylon net bags served as controls.

Samples were taken every 2 weeks and examined as in 2.5.1 above. Recovery of P. meadii was attempted both on P10VP medium and by baiting with tender rubber petioles.

3. Results

3.1 Survival of mycelium in soil

In the main experiment mycelial mats showed signs of rapid lysis after one week. Vacuolation, granulation of cytoplasm and disintegration of hyphae were frequently observed (Fig. 23). Both in

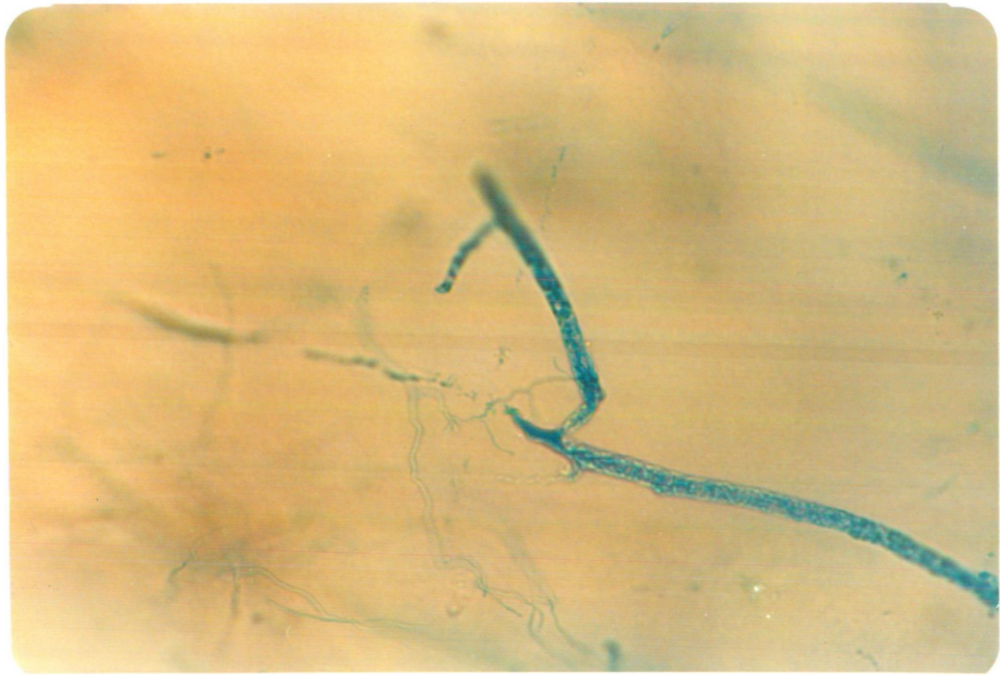


Fig. 23 Mycelium of P. meadii disintegrating in the first week after incubation in wet soil. Note normal hyphae with contents, hyphae showing granulation of contents and disintegrating mycelia.

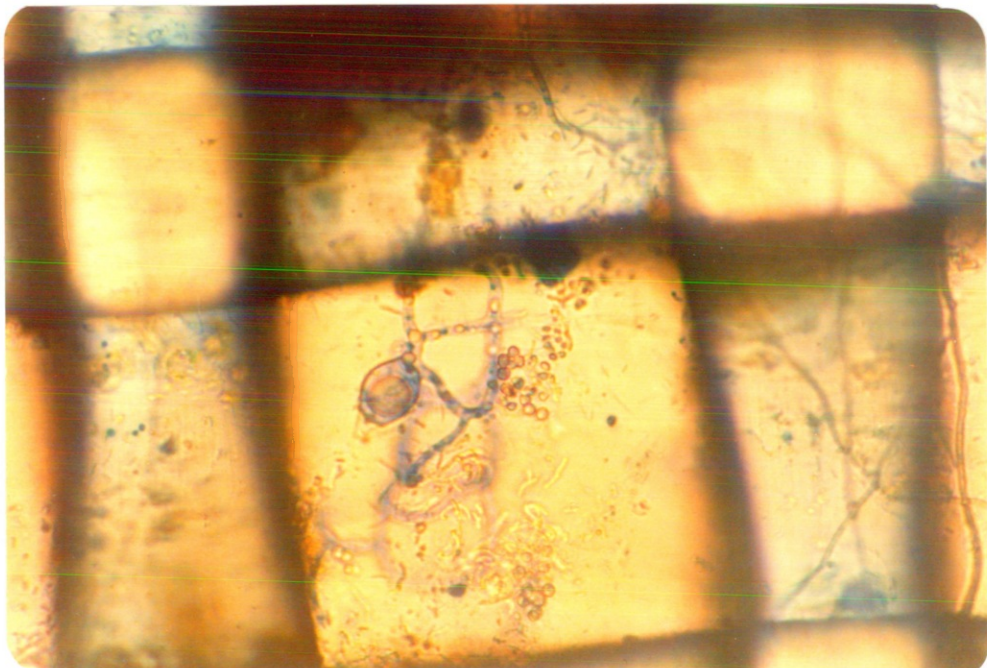


Fig. 24. Presence of bacteria in association with lysed hyphae of P. meadii in wet soil. Note the disintegration of sporangia that were formed in the first week.

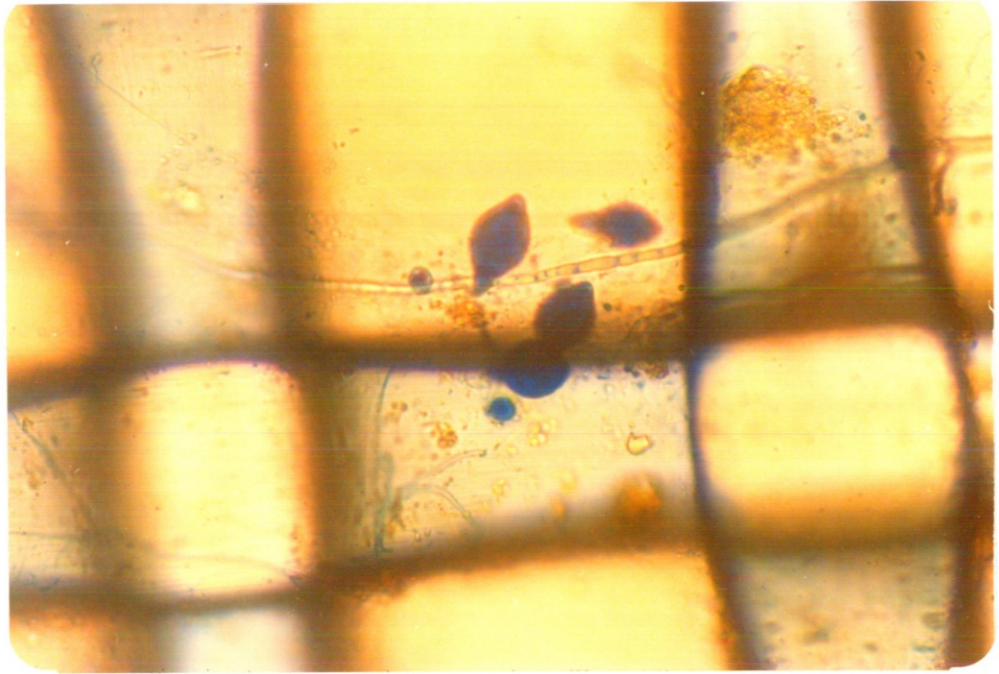


Fig. 25. Mycelium of *P. meadii* showing vacuolation of mycelium, production of sporangia and small chlamydospores first week after incubation in wet soil.

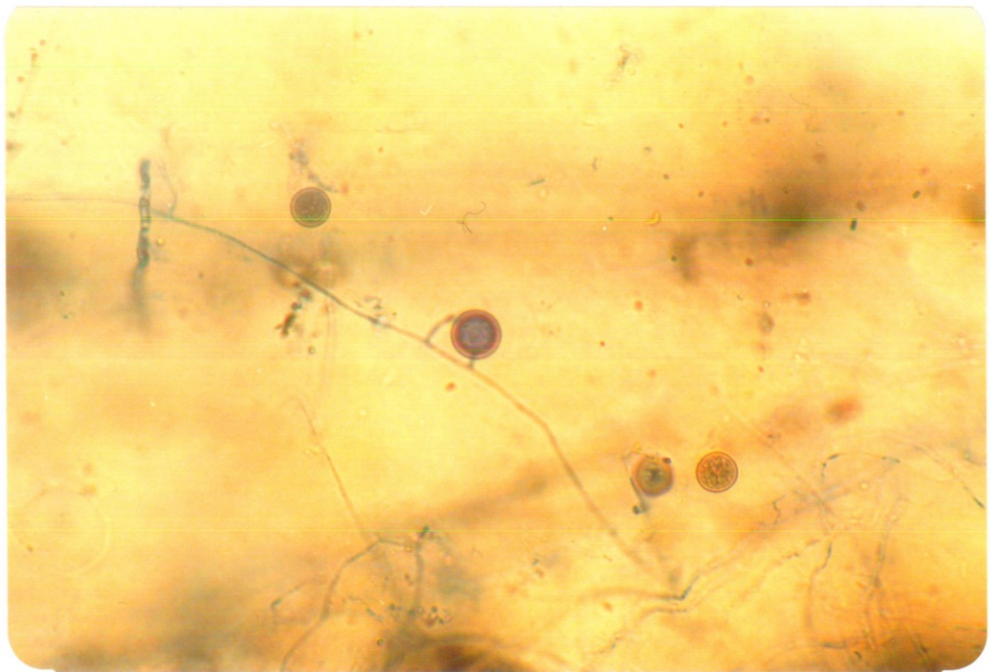


Fig. 26. Production of chlamydospores from mycelium in the 1st week. Note the disintegrating hyphae and the increase in size of chlamydospores,

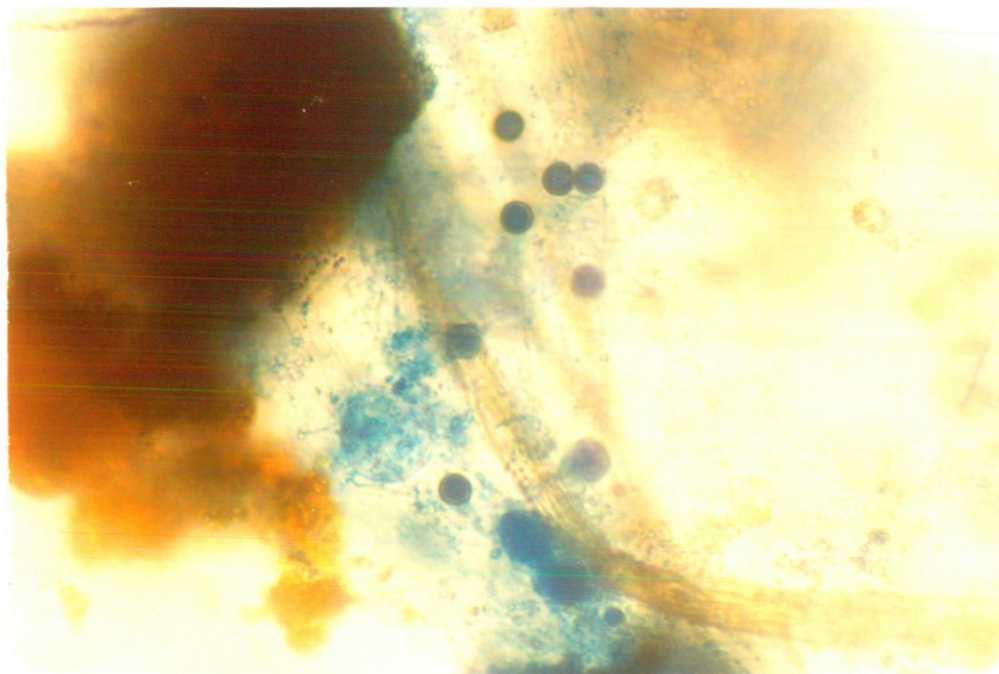


Fig. 27. Mycelium of P. meadii almost totally disintegrated in soil 14 days after incubation in wet soil. Note the presence of many chlamydospores.

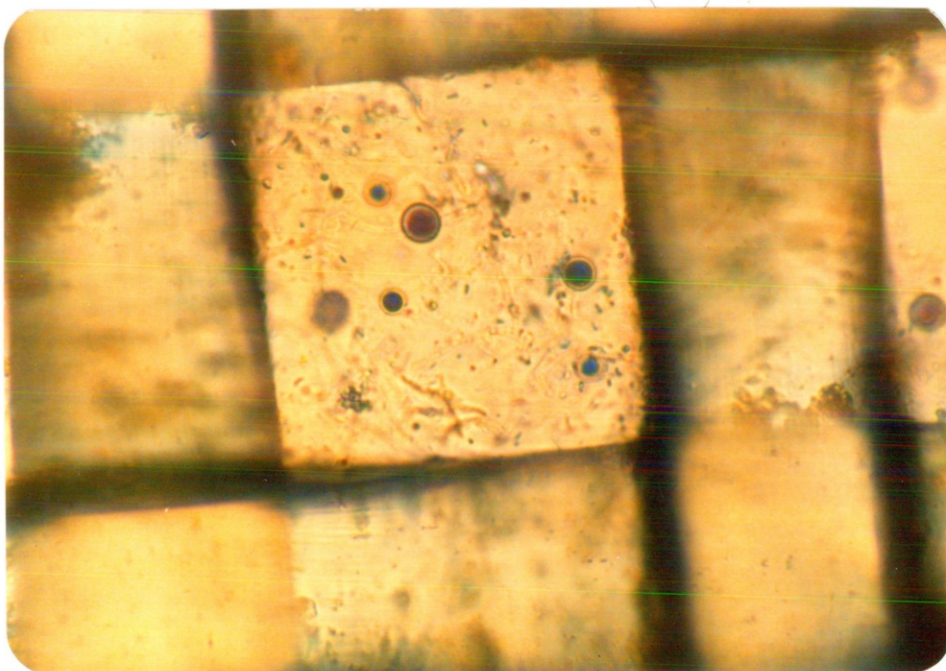


Fig. 28. Mycelium of P. meadii not present in soil 21 days after incubation in dry soil. Note the reduction in the number of chlamydospores.

soil maintained at 50% MHC (wet) and soil allowed to dry periodically and then wetted (dry), lysis was virtually complete after 3 weeks (Table 32). Many bacteria were seen in association with the lysed hyphae (Fig. 24). Chlamydospores had formed in both soils after one week (Fig. 25) and increased in size and number (Figs. 26 & 27) but the numbers declined thereafter (Fig. 28). Very few sporangia were observed and those only in wet soil during the first two weeks (Fig. 25). Cultures of P. meadii were obtained upto 3 weeks but the % recovery declined markedly over that period. No cultures were obtained in week 4 despite the apparent presence of chlamydospores (Table 32).

The results of the subsidiary experiment (Table 33) generally compared with those of the main experiment. Some lysis of mycelium was observed even after 1 day and by the fifth day approximately 50% had been lysed, with no apparent difference between soils of different moisture content. Chlamydospores formed in both soils from day 3 but were more abundant in the soil sample at 50% MHC. A few sporangia formed in moist soil from day 2 onwards but none in dry soil.

3.2 Survival of sporangia in soil

Sporangia germinated within 24h either directly or by producing a new sporangium at the end of a germ tube (Fig. 29). The latter method of germination was more frequently observed in dry soil. By 48h sporangia had become distorted, often elongated. In some the contents had divided to form zoospores (Fig. 30). Although release was not observed, some zoospores were seen on the mesh pieces (Fig. 31). After 48h sporangia began to disintegrate and most were without contents by day 3 though in a few the remains of the cytoplasm could be seen. Mycelium from germinated sporangia had also begun to lyse (Fig. 32) but some germinated zoospores with germ tubes were seen (Fig. 33).

In the second week apparently healthy sporangia which stained deeply

Table 32. Observations on mycelial mats of P. meadii buried in soil for 4 weeks.

Observation	Soil moisture ^a	Week No.			
		1	2	3	4
Mean % lysis of mycelium ^b	Wet	50	50	90	100
	Dry	50	60	100	100
Numbers of chlamydospores ^b	Wet	19	25	17	16
	Dry	25	19	12	14
Numbers of Sporangia ^b	Wet	2	1	0	0
	Dry	0	0	0	0
% Recovery on P10VP medium ^c	Wet	56	42	7	0
	Dry	31	29	1	0

^a Soil at 50% MHC in sealed (Wet) or unsealed (Dry) petri plates.

^b Based on an examination of 60 squares in each of three pieces of nylon mesh. Numbers of chlamydospores and sporangia are the means for the three nylon pieces (i.e. mean no./60 squares)

^c Each value is based on 30 isolations.

For further details, see text.

Table 33. Observations on mycelial mats of P. meadii buried in soil for one week.

Observation	Soil moisture ^a	Day no.					
		1	2	3	4	5	6
Mean % lysis of mycelium	Wet	10	20	20	30	50	45
	Dry	20	30	30	40	60	55
Numbers of chlamydospores ^b	Wet	0	0	6	11	23	24
	Dry	0	0	2	10	7	9
Numbers of sporangia ^b	Wet	0	2	3	5	7	0
	Dry	0	0	0	0	0	0

^a Soil at 50% MHC in sealed petri dishes (Wet) or air-dried soil in unsealed dishes (Dry).

^b Based on an examination of 10 squares in one piece of nylon mesh.

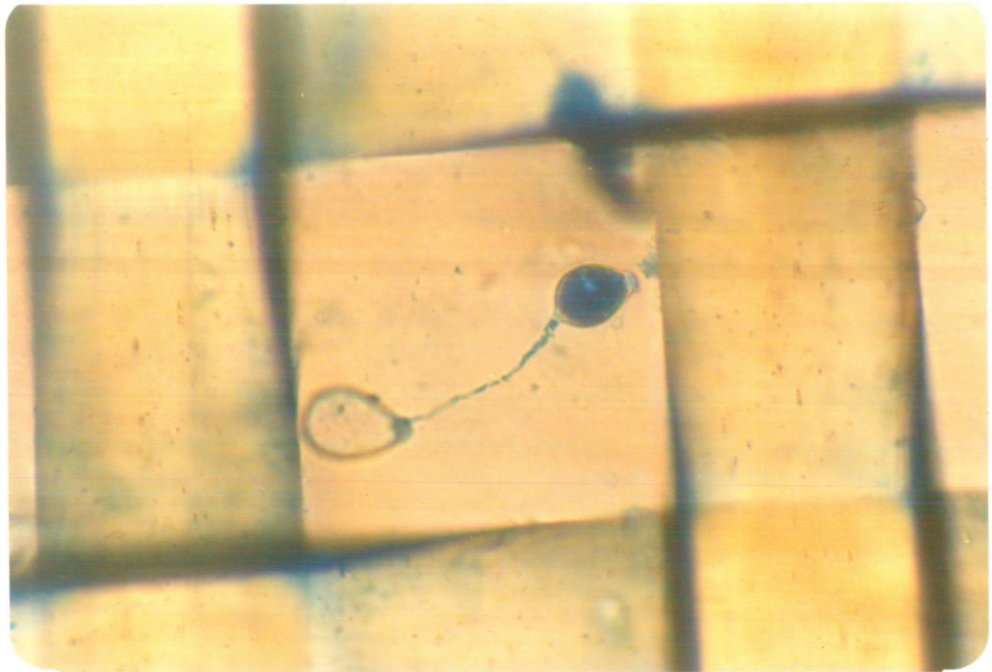


Fig. 29. Sporangium of *P. meadii* producing a secondary sporangium at the end of a germ tube, 24h after incubation in soil.

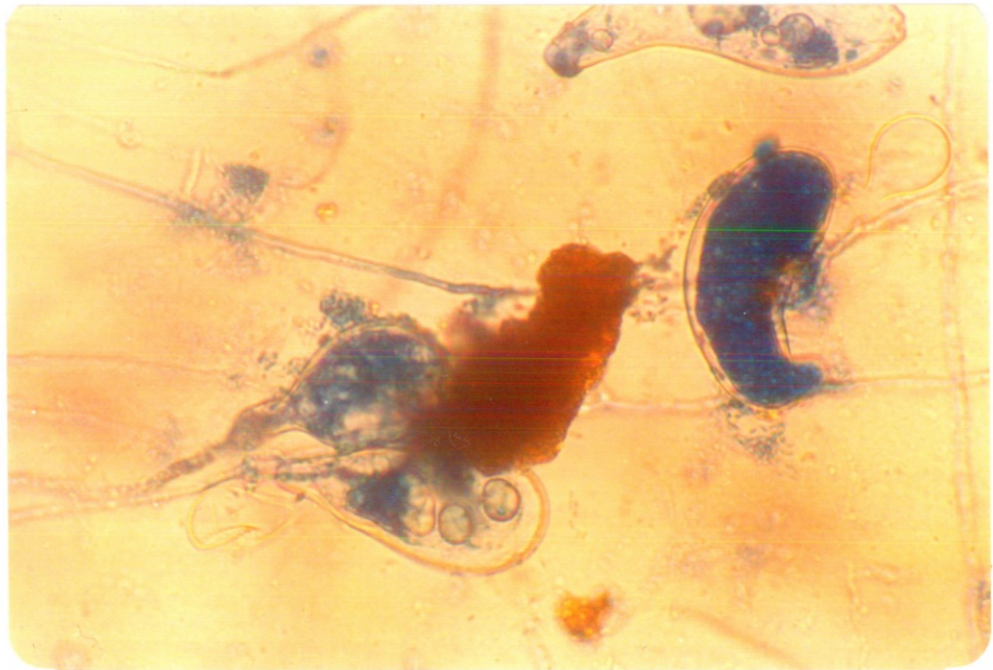


Fig. 30. Sporangia of *P. meadii* with zoospores after 24h in soil. Note also, direct germination of sporangia and the presence of long strands of mycelium.

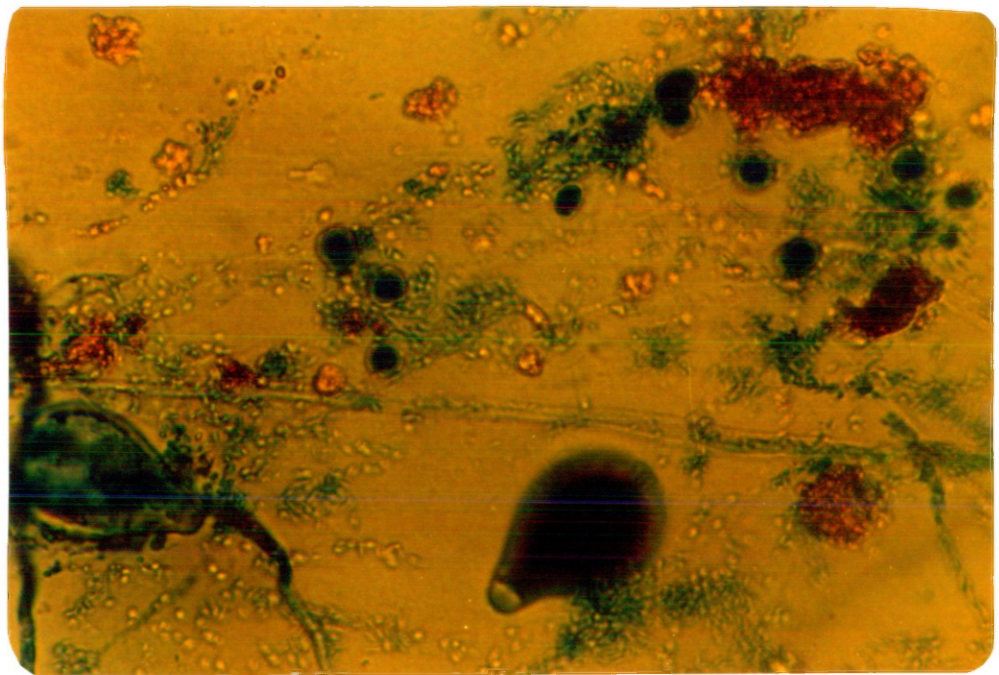


Fig. 31. Sporangia of *P. meadii* after 48h in soil. Some sporangia have germinated directly and there appear to be encysted zoospores.

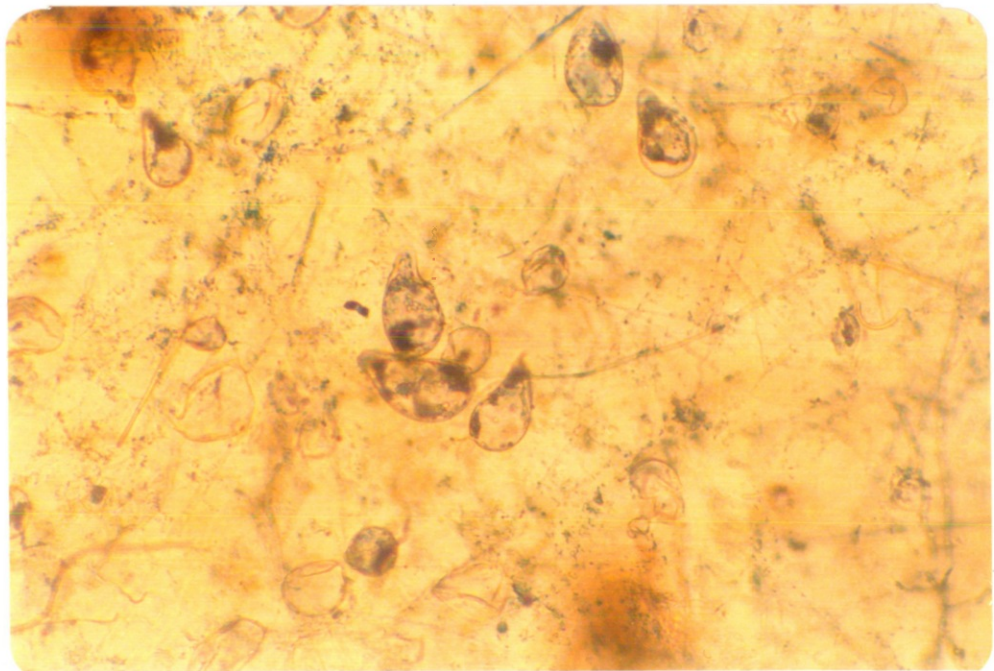


Fig. 32. Sporangia of *P. meadii* in soil 72h after incubation. Note the shrunken cytoplasm, empty sporangial cysts and disintegrated mycelia.

were observed in both the wet and dry soil samples, but more abundantly in wet soil (Fig. 34). Possibly these were sporangia that were newly formed from mycelium produced by the first crop of sporangia. While some mycelial fragments and sporangia were seen in week 3, none were detected in weeks 4 and 5. Correspondingly, in these weeks no cultures of P. meadii could be recovered by plating pieces of nylon mesh on P10VP medium. In the previous 3 weeks, recovery had dropped from between 73 and 82% to 25-35% (Table 34).

3.3 Survival of chlamydospores in soil

The numbers of chlamydospores declined in both wet and dry soil (Fig. 35) and Table 35) until by 18 weeks none could be found. Although germination of chlamydospores was rarely seen (Fig. 36) some apparently remained viable because cultures of P. meadii were obtained to week 12 by plating mesh pieces buried in wet soil on P10VP medium and to 8 weeks from mesh pieces buried in dry soil (Table 35).

3.4 Detection of P. meadii in soils from infected rubber plantations.

At the beginning of the experiment, healthy green pods were present on trees and leaf fall was not observed. Heavy or intermitten rain prevailed throughout the experimental period except for a short dry period at the beginning of August 1985. The conditions therefore, were conducive for Phytophthora infection. Pod infection and the leaf fall phase of the disease developed during the experimental period but was not severe. Soil was baited in cocoa pods at the time pod infection appeared in the field. A 44% recovery was obtained at this period and the % recovery increased as the disease increased, reaching 56% during the peak disease period (Table 36). As pod infection and leaf fall tailed-off recovery by this method was not possible. On the selective medium a low percentage recovery (10%) was obtained even before the infection was observed on rubber pods or leaf fall detected. However, an increase in

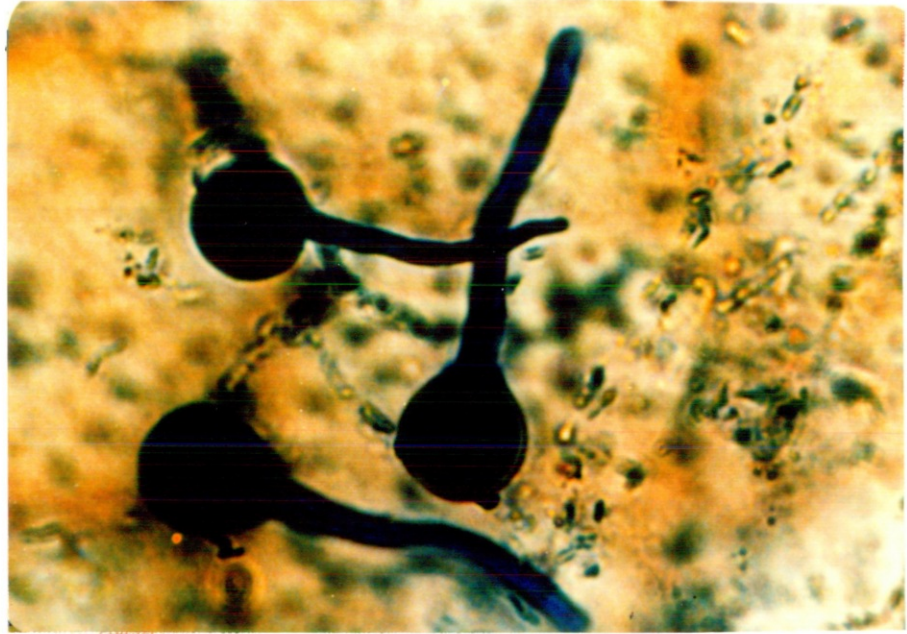


Fig. 33. Germinated zoospores 3 days after incubating sporangia in wet soil (x 5000).

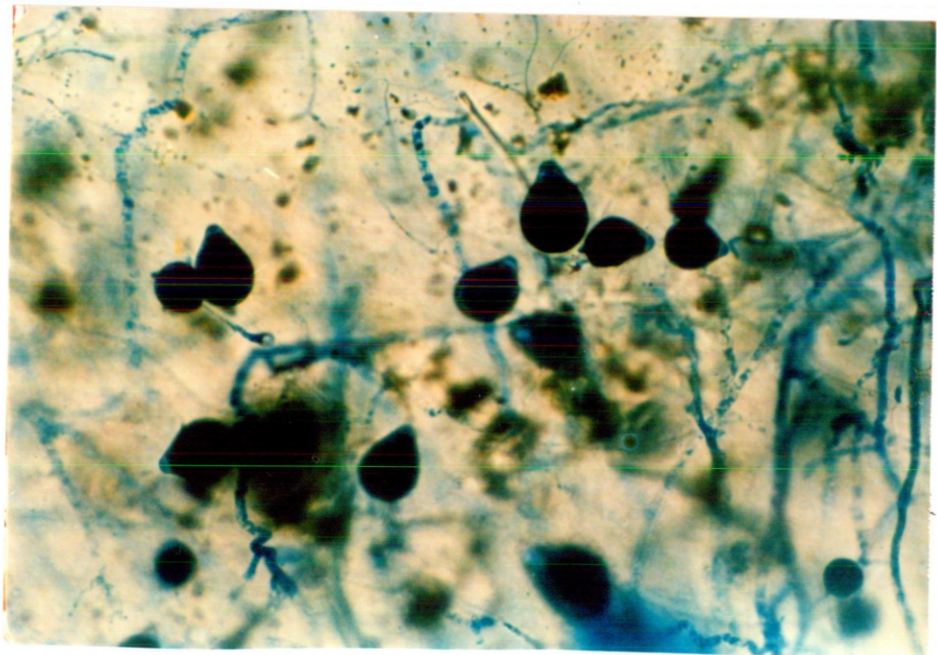


Fig. 34. Sporangia in soil after 14 days.

Table 34. Recovery of Phytophthora meadii from sporangia incubated in soil.

Period after incubation (wk)	% Recovery ^a	
	Wet soil	Dry soil
1	82	73
2	63	83
3	35	25
4	0	0
5	0	0

^a Each value based on 30 isolations on P10VP medium.

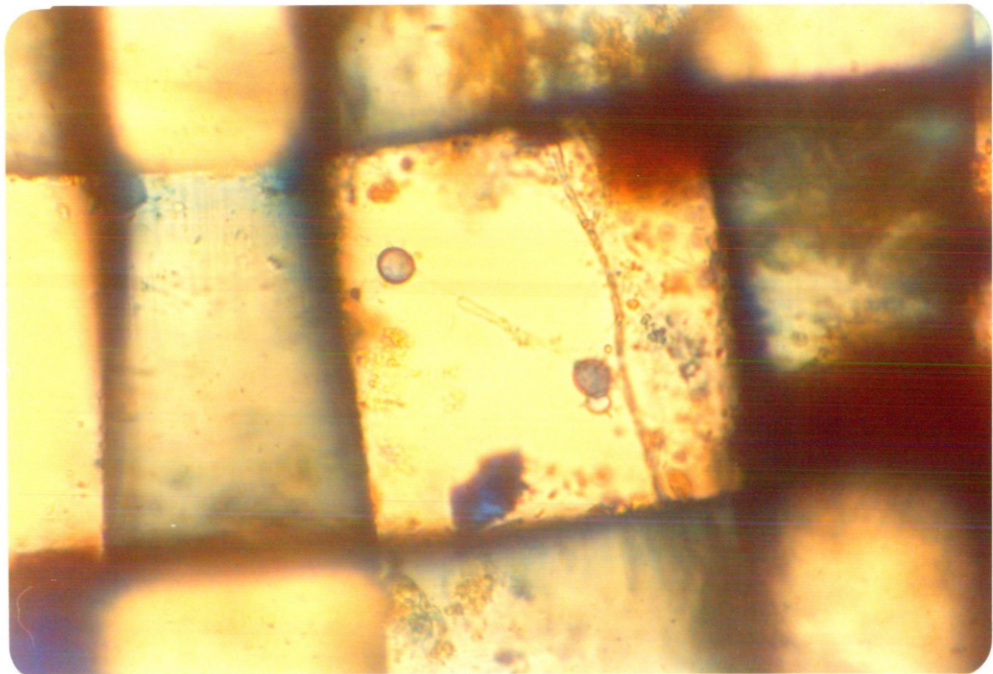


Fig. 35. Chlamydospores after 4 months in soil.

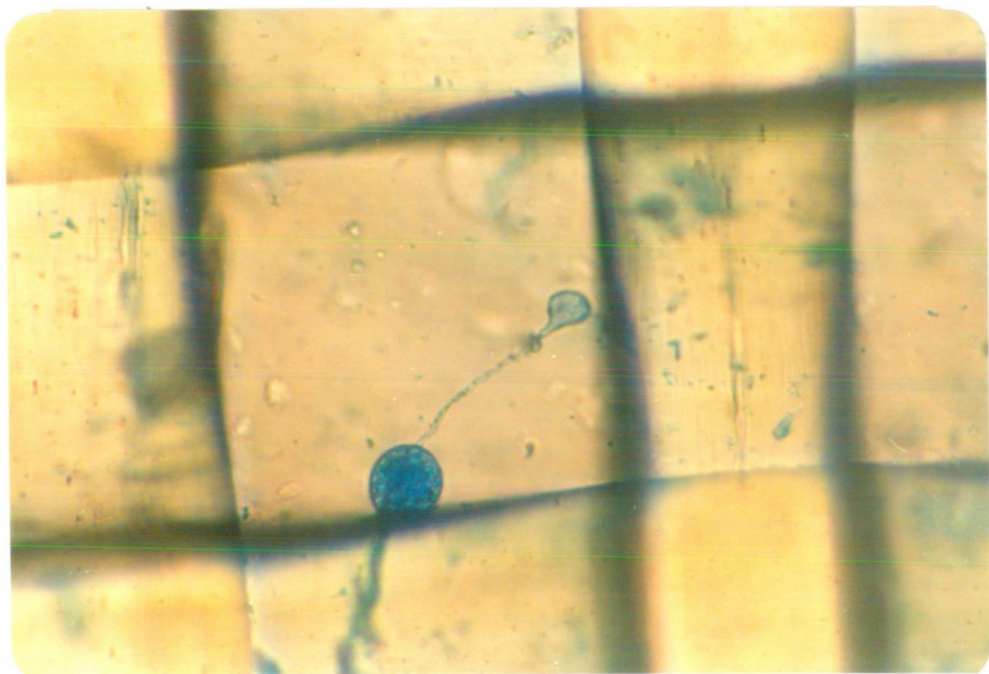


Fig. 36. Germination of a chlamydospore of *P. meadii* in soil, 3 weeks after incubation. (This was rarely observed).

recovery was observed throughout the disease period particularly when the pods were actively producing sporangia (Table 36). It was possible to isolate P. meadii over a longer period by using P10VP medium than by baiting with cocoa pods. After the rubber pods dried up and fell to the ground a low percentage of recovery (10%-20%) was obtained and subsequently it was not possible to recover the fungus even on P10VP medium.

3.5 Survival in infected rubber petioles in soil.

3.5.1 Laboratory tests

When examined after 2 weeks the entire length of the petioles had turned black. There were abundant intracellular mycelium in the tissues and many thin-walled chlamydospores, often several in a single cell and most of them small (c. 10 μ m) (Fig. 37). By week 10 all the mycelium had lysed but the numbers of chlamydospores increased to week 12. This increase was greatest in soils at 20°C (Table 37, Fig. 38). In wet soil at 20° thick-walled chlamydospores were observed from week 12 but somewhat later (16 or 20 weeks) in other treatments (Fig. 39). Numbers of thin-walled chlamydospores declined drastically during the period, week 12 to week 24, particularly at 25° and 28°C. At 20°C the decline was more gradual. During this period and to week 28, the numbers of thick-walled chlamydospores increased slightly in most treatments but they were never abundant. Subsequently, the numbers of these chlamydospores also declined. By week 40 the petioles had largely disintegrated. Few chlamydospores were seen though some were large and appeared to have a thick wall with several layers (Fig. 40, Table 37).

There was a 100% recovery of P. meadii from all treatments to the 4th week (Table 38). The recovery rate then declined sharply to week 10. Thereafter it remained at a low level (c. 10-20%) in most treatments, slightly higher (c. 20-30%) at 20°C, until week 22. At week

Table 35. Observations of chlamydospores of *P. meadii* buried in soil.

Observation	Soil	Week no.						
		moisture ^a	1	4	8	12	16	18
Number of chlamydospores ^b	Wet	83	49	27	8	6	0	0
	Dry	80	53	24	12	5	0	0
% Recovery on P10VP medium ^c	Wet	50	23	8	3	0	0	0
	Dry	39	16	9	0	0	0	0

^a Soil at 50% MHC in sealed (Wet) or unsealed (Dry) petri dishes.

^b Based on an examination of 60 squares in each of three pieces of nylon mesh. Values are mean numbers/60 squares.

^c Each value is based on 30 isolations.

Table 36. Detection of P. meadii in soil collected from a rubber plantation.

Date (1985)	Field conditions			% Recovery	
	Rain	Pod infection ^a	Leaf fall	Baiting	P10VP
April 2	Heavy	-	-	NA	10
15	Heavy	-	-	NA	10
29	Heavy	-	-	NA	0
May 13	Heavy	+	-	44	30
27	Moderate	+	-	44	40
June 10	Moderate	++	-	38	50
24	Moderate	++	+	56	70
July 8	No rain	+	+	44	60
20	Intermittent	+	+	0	50
Aug 5	Moderate	Pods	+	0	10
19	Moderate	dried	+	0	20
26	No rain	fallen	-	0	0
Sep 9	No rain	fallen	-	0	0
23	No rain	fallen	-	0	0
Oct 7	Moderate	fallen	-	0	0

^a Pod infection/Leaf fall absent (-), present (+) or severe (++)

NA Data not available

Table 37 Production of chlamydospores in infected rubber petioles incubated in dry and wet soil at 28°, 25° and 20° (Laboratory tests).

Period after incubation (Weeks)	No. chlamydospores ^a					
	28°C		25°C		20°C	
	wet ^b	dry ^c	wet	dry	wet	dry
2	6	3	7	9	9	8
4	22	27	23	27	49	47
8	19	18	20	25	51	50
12	34(21)	22	21	40	41	53
16	26(19)	18	24(16)	31(8)	33	29
20	21(5)	14(8)	11(8)	19(9)	42(6)	37(16)
24	12(8)	21(18)	5(3)	10(8)	39(12)	41(22)
28	6(3)	12(10)	9(6)	16(12)	21(16)	26(13)
32	9(7)	16(9)	9(5)	12(10)	16(8)	17(10)
36	11(8)	10(7)	11(11)	9(9)	10(6)	9(7)
40	4(3)	7(5)	1(1)	2(2)	4(2)	3(2)

^a Values are total counts in 10 fields at x400.

Number of thick-walled chlamydospores in parentheses

^b Wet = 50% MHC

^c Dry = Soil allowed to dry periodically.

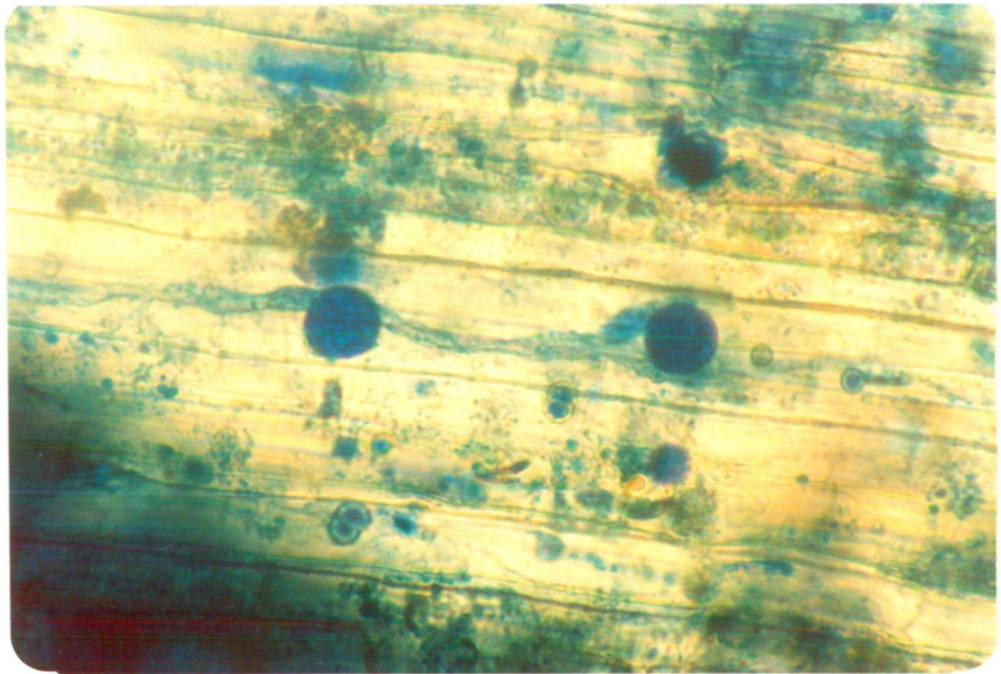


Fig. 37. Intra-cellular mycelium and thin walled chlamydospores in infected rubber petiole tissue, incubated in soil for two weeks.

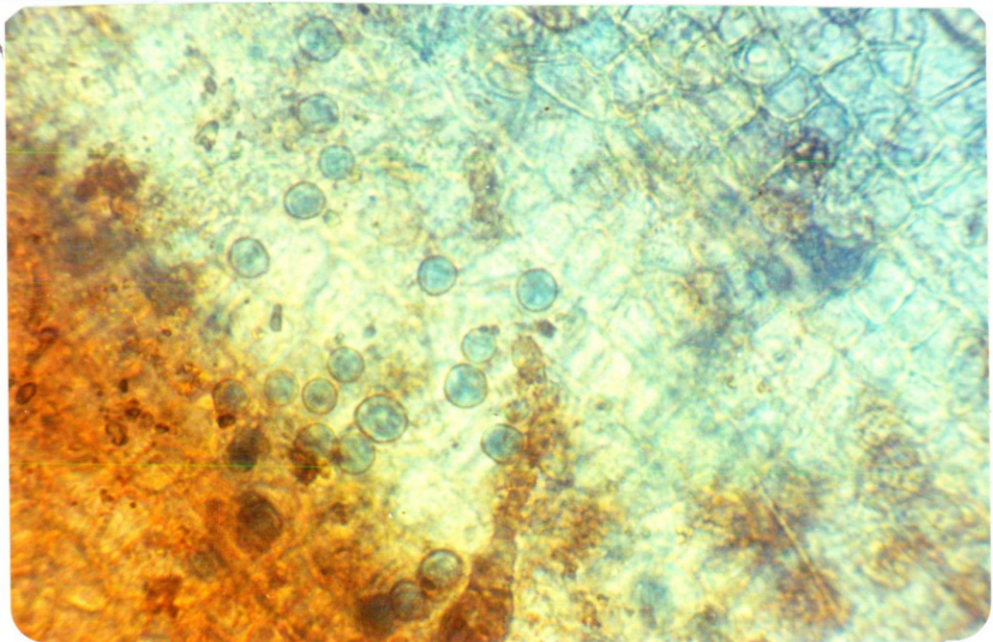


Fig. 38. A large number of chlamydospores in infected rubber petiole tissue, incubated in soil for 10 weeks.

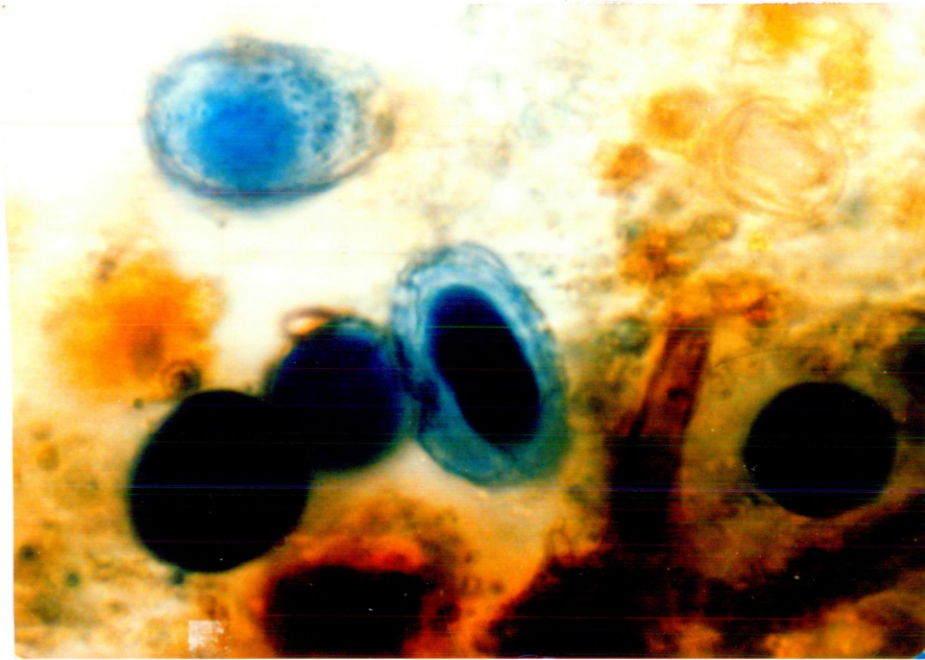


Fig. 39. Chlamydospores in petiole tissue incubated in soil for 16 weeks. Note the thick and the thin-walled chlamydospores.

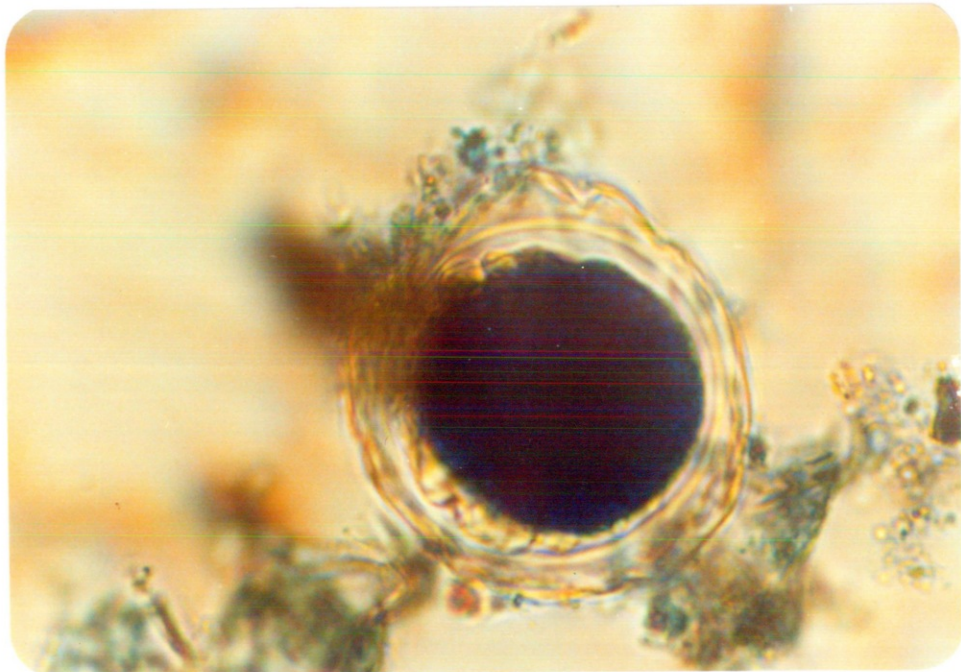


Fig. 40. Chlamydospores in rubber petiole tissue after 40 weeks of incubation in soil. Note the appearance of a several layered wall.

Table 38. Recovery of Phytophthora fungus from infected petioles incubated in soil at 28^o, 25^o and 20^oC (Laboratory tests).

Period after incubation (Weeks)	% Recovery ^a on P10VP medium					
	28 ^o C		25 ^o C		20 ^o C	
	wet ^b	dry ^c	wet ^b	dry ^c	wet ^b	dry ^c
2	100	85	100	100	100	100
4	100	100	100	100	100	100
6	45	60	60	90	100	60
8	60	35	40	25	70	60
10	15	20	25	40	25	40
12	10	35	25	15	50	45
14	15	25	15	25	35	25
16	15	10	20	20	20	30
18	10	—	10	5	30	30
20	15	10	10	15	—	40
22	10	20	20	5	10	15
24	0	5	10	0	0	0
26	0	0	0	0	0	0
28	0	0	0	0	0	0
30	0	0	0	0	0	0

^a Each value based on 20 isolations

^b Wet = 50% MHC

^c Dry = soil allowed to dry periodically

— data not available

24 recovery of P. meadii was poor and there were no successful isolations beyond this even though some chlamydospores were apparently present.

No sporangia or oogonia were observed throughout the experiment nor were chlamydospores seen in the healthy petioles which were examined periodically.

3.5.2 Field tests

As in the laboratory tests, intracellular mycelium and thin-walled chlamydospores were seen in petioles 2 weeks after placing them in soil, though there were relatively few chlamydospores at this stage. By week 4 most mycelium had begun to lyse and none was seen by week 6. During this period the numbers of chlamydospores increased and continued to do so to week 12 (Table 39, Fig. 41). At this stage, thick-walled chlamydospores were also seen in the tissues (Fig. 42). Thereafter the numbers of chlamydospores declined but most of those present had extremely thick walls.

Generally, P. meadii was recovered more readily on P10VP medium than by baiting (Table 39). By week 10 there was only a 10% recovery via rubber petioles and no isolates were obtained by this method after week 14. Recovery of P10VP medium was fairly high to week 14 (50% or above) but then declined rapidly over the next 2 weeks. After 20 weeks no isolates were obtained on this medium. Chlamydospores were present at this time but were mostly thick-walled and presumably these are either difficult to germinate or they have lost viability.

By week 20, most petioles had decayed. The remainder had only the outer tissues intact; the central core had decayed completely. Generally, decay was more rapid in the field than in the laboratory tests and in the later stages the nylon bags were penetrated by roots of the cover crop (Pueraria phaseoloides). No chlamydospores of P. meadii were seen in the healthy petioles which decayed completely some 3 weeks later than the infected ones.

Table 39. Production of chlamydospores and recovery of P. meadii from infected rubber petioles incubated in soil under field conditions.

Period after incubation (Weeks)	No Chlamydospores ^a	% recovery of fungus	
		Baiting ^b	P10VP medium ^c
2	4	70	90
4	18	45	100
6	20	60	80
8	31	50	50
10	36	10	70
12	39(18)	0	50
14	-	10	60
16	24(20)	0	30
18	20(15)	0	10
20	13(10)	0	0
22	6(4)	0	0
24	10(9)	0	0
26-30	0	0	0

^a Values are total counts in 10 microscopic fields at x 400.

Number of thick-walled chlamydospores in parentheses.

^b Each value based on infection on 20 pieces rubber petiole.

^c Each value based on 20 isolations

- Data not available.

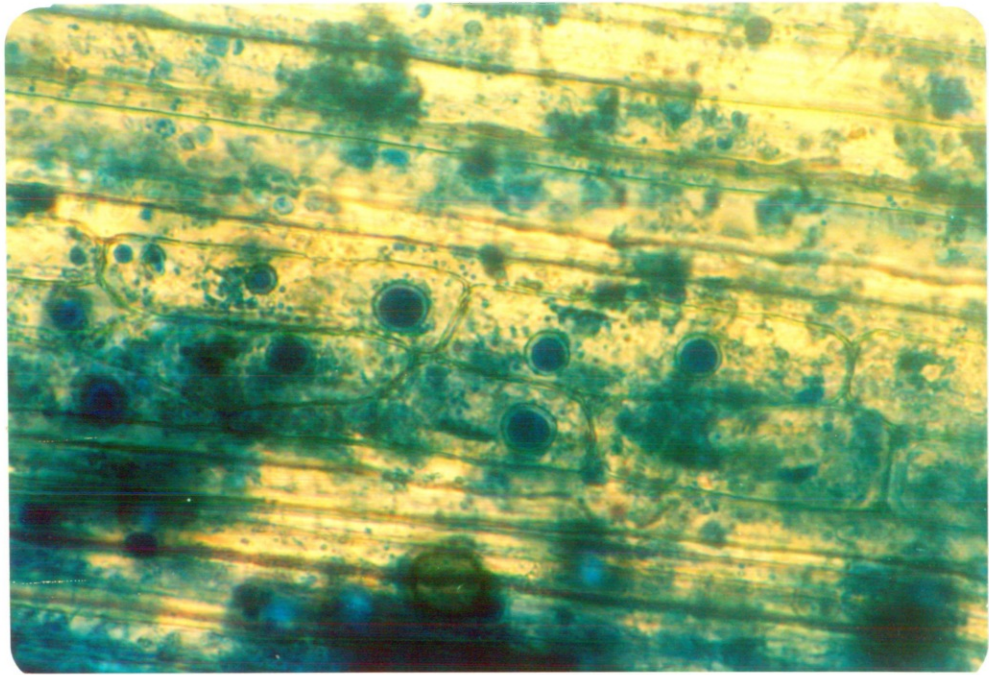


Fig.41. Production of chlamydospores in infected petioles of rubber buried in field soil, for six weeks.

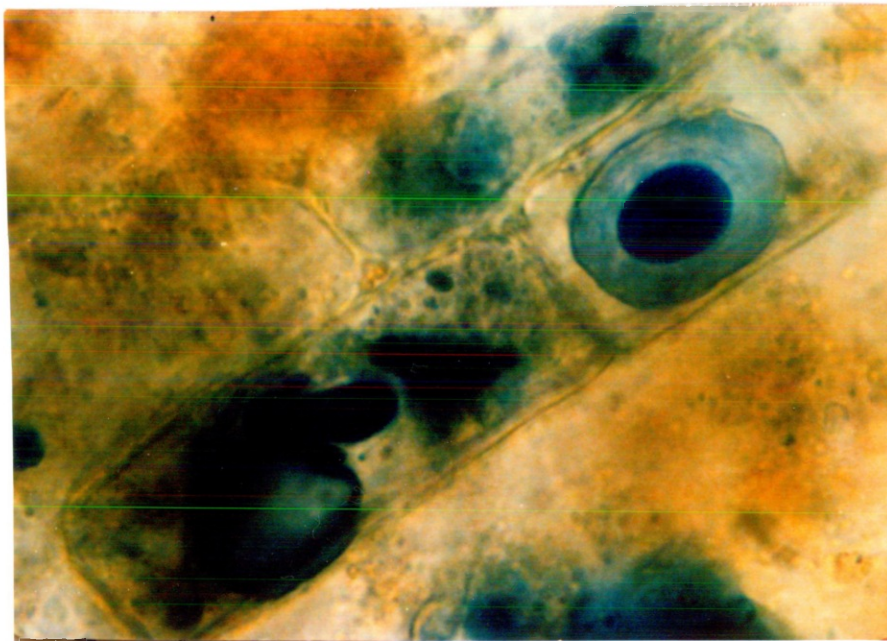


Fig. 42. Thick-walled chlamydospores produced in infected petioles placed in field soil, for 16 weeks.

Discussion

Recovery of P. meadii from soil in a rubber plantation (Table 36) was clearly associated with infection in the trees, notably on pods. Failure to isolate the fungus when the pods had dried suggests that a continuous supply of sporangia, washed off from the pods by rain is necessary to maintain this soil inoculum. It suggests further that the recovery of the fungus from soil in April may have been associated with pod infection which was not obvious and which remained undetected. The evidence from the laboratory tests indicate that sporangia survive only for about 3 weeks in soil (Table 34) and this corresponds well to the recovery of the fungus from field soil for 3 weeks in August when most pods had dried and sporangia are not available on the pod surface.

The laboratory tests also indicated that mycelium was soon lysed in soil whether this was kept at 50% MHC or whether it was subjected to wetting or drying (Table 32 and 33). Lysis of mycelium was complete in 3 weeks in dry and in 4 weeks in the wet soils. A high percentage of recovery of the fungus was obtained in the first 2 weeks indicating that the mycelium could act as infective propagules only for a short time. Dry conditions suppressed the recovery rate and this is particularly important because under natural conditions wet and dry periods occur which means that the mycelium is likely to be destroyed under such conditions than during a continuous wet period. Mycelium in petioles was also relatively short-lived once these had been buried in soils. Thus it seems unlikely that survival by mycelium or sporangia plays any significant role in the epidemiology of the disease caused by P. meadii. Nor apparently do oogonia, for none were seen. However, this was to be expected because the isolate was of the A₁ compatibility type which appears to predominate in the rubber plantations of Sri Lanka and this does not respond to stimulation by Trichoderma species.

On the evidence available from this investigation, survival of P. meadii by chlamydospores would seem most likely. In the laboratory tests where these were produced in culture and then added to soil, numbers declined rapidly but some were present 16 weeks later. Also, the low temperatures and dry condition of the soil promoted slightly more chlamydospore formation in infected petioles and also a better recovery of the fungus. This is particularly important for areas where the temperature could be low, especially at high altitudes.

When infected petioles were buried in soil, both in the laboratory and in the field, chlamydospores formed after 2 weeks and some could be detected in the decaying tissues to 40 weeks in the laboratory (Table 37) and 24 weeks in the field (Table 39). When the disease is severe on trees many infected leaves fall and remain as groundlitter until they are decayed so the survival of the fungus in petioles as chlamydospores could be particularly important.

The rapid decay of petioles in the field is understandable due to the vast number of activities that go on in the soil under natural conditions. The activities of earthworms, termites and other types of living organisms : on the organic matter in the petioles may have contributed to this rapid decay. Although simulated experiments contribute a wealth of information, the importance of carrying out research on survival aspects under actual field conditions should be emphasized in the light of these studies.

Most chlamydospores which survived for long periods were thick-walled and failure to obtain cultures of P. meadii when these predominated possibly indicates that suitable conditions for their germination were not provided by the isolation medium. In view of their likely role in the perennation of the fungus, these thick-walled chlamydospores merit further investigation. First, techniques need to be devised to produce

them in quantity for experiments. This might be attempted initially through the use of infected petioles and then extracting them. Alternatively, thin-wall chlamydospores, produced in culture, either in CaCO_3 broth or petiole extract, might be induced to become thick-walled by subjecting them to soil extracts sterilized by filtration through membranes. The response of these chlamydospores to roots of rubber would then be of particular interest.

There is at least some circumstantial evidence that soil-borne inoculum may be important in Sri Lanka. During the North-East monsoon (September - November) some infection of tapping panels has been noted especially on those nearest the ground. At this time the disease is not present on the leaves or pods. It is therefore likely that such infection could arise from the inoculum present in the soil through the decay of petioles that had fallen during the previous disease season (July - August).

CHAPTER 4

THE USE OF FUNGICIDES TO CONTROL PHYTOPHTHORA DISEASES
IN RUBBER

1. Introduction

The application of fungicides as preventive and curative measures for the control of bark rot caused by Phytophthora spp. started in 1916 (Petch, 1921) and has been used extensively since the mid 1940s. Today most estates use water miscible fungicides to protect the tapping panels from May to October when the disease is most severe. The current recommendation is to apply the fungicide on the tapping cut each day when latex is collected. To prevent wash off of the fungicide by frequent rains, tapping cuts are treated with a water proof panel dressing at fortnightly or monthly intervals.

The fungicides available for use, both as prophylactic and curative measures are Antimucin (phenyl mercuric acetate) and Fylomac (tetradecylpyridinium bromide). Of the two, Antimucin is the more popular because it is effective at comparatively low concentrations and is cheaper. However, the need to apply these materials daily is inconvenient and increases cost. Also, results under field conditions are often inconsistent and unsatisfactory. This is particularly due to the fungicide being washed away by the frequent rains. It would therefore, be very useful if a systemic fungicide were effective against Phytophthora spp. in rubber, particularly as a panel treatment for bark rot or as a foliar spray against shoot die-back in seedling nurseries or young plantations where spraying is feasible.

To achieve maximum information in a short period, this study was

limited to three aspects:

- (1) Six candidate systemic fungicides were tested in the laboratory to determine their toxicity to five Phytophthora species attacking rubber.
- (2) Three systemic fungicides Ridomil, Aliette and Difolatan, already recommended for control of other Phytophthora diseases were compared with Antimucin. A new formulation Oxadixyl (code no. SAN 371 F4 PA09) supplied by the Sandoz Company was also tested in this experiment to determine its efficacy as a prophylactic fungicide for panel treatment, using budded nursery plants.
- (3) Infected, mature rubber trees were treated with Ridomil, Aliette, Difolatan, Oxadixyl or Antimucin to compare their efficacy in controlling Phytophthora panel disease, under field conditions.

2. Literature review: Developments in chemical control.

Over the past 10 years, several chemicals with novel features for the control of diseases caused by Phytophthora species, have been formulated and marketed. Four groups are reported to be active against Oomycetes (Schwinn, 1979): the carbomates, acetamides, acylalanines and metal ethylphosphanates.

Of these, acylalanines have high activity both in-vitro and in-vivo and act on most species of this genus. They have strong protective and curative activity, are taken up rapidly from foliage, shoots and roots and translocated upward within the plant. So far the most effective molecule in this class is metalaxyl. This can be applied as a foliar spray at low rates and as a soil or seed treatment to control stem and root diseases such as tobacco black shank caused by P. nicotianae var. nicotianae (Kannwischer & Mitchell, 1978; Mortan et al., 1978).

Metalaxyl inhibits formation of secondary haustoria and mycelial growth inside the leaf, formation of lesions and sporulation.

Milfuram (patafol) has basically similar properties but is less active in its overall performance. However, it is reported to be effective against P. infestans (late blight) on potato (Hartill, 1980; Cooke et al., 1981); P. parasitica (foot rot) on citrus (Timmer, 1979) and P. megaspermae var. sojae (seedlings rot) on soybean (Lazarovite et al., 1980).

Ethyl phosphonates represent a new type of chemical with high fungitoxic activity on most species in the Peronosporales. The most effective compound within this class is phosethyl Al which was described by William et al. (1977). However, some Phytophthora species such as P. infestans are relatively insensitive to it.

Phosethyl aluminium or Aliette is the first commercial fungicide that is truly systemic. It is translocated by both acropetal and basipetal means after foliar application (Bertrand et al., 1977 cited by Schwinn, 1983). This results in curative action and leads to temporary protection of new leaves formed after application. In a wide variety of crops such as avocado, pineapple and certain ornamentals, basipetal transport after foliar application is sufficient to fully protect the root system and the stem base against attack by soil borne Phytophthora spp. such as P. cinnamomi and P. nicotianae var. parasitica (Fariah et al., 1981).

Carbamates are mainly active as soil treatments against root and stem diseases caused by Phytophthora and Pythium spp. in ornamentals (Englander et al., 1980).

Acetamides have selective activity against fungi in the Peronosporales. For example P. infestans is more than 100 times more sensitive in vitro than other Phytophthora spp. (Richardson, 1976).

Therefore, late blight of potato and tomato is the only disease caused by a Phytophthora sp. against which an acetamide (cymoxanil) is recommended (Douchet et al., 1977).

Some pathogens have developed resistance to these newer fungicides. Metalaxyl-resistant strains of P. infestans on potato (Davidse et al., 1981) and of Peronospora tabacina on tobacco (Bruck et al., 1981) have already occurred in the field. Thus the acylalanines practically have been found to be more useful in mixtures with other fungicides such as dithiocarbomates and phthalimides (Urech et al., 1977). Such mixtures also improve disease control on senescing foliage on which acylalanines are less effective than on young tissue.

Field trials on rubber, in Malaysia for controlling panel disease have indicated that Ridomil at 0.2% a.i. is slightly better than Difolatan at 0.8% a.i., both as a protectant and curative fungicide (Tan, 1983).

3. Materials and Methods

3.1 Effect of fungicides on mycelial growth of Phytophthora species

The following fungicides were tested:

- (a) Mancozeb (Dithane 945)
- (b) Tridemorph + maneb (Tridemorph 11% + Maneb 36%)
- (c) Metiram + ofurace (Metiram 15% and 50% ofurace)
- (d) Recoil (Oxadixyl 10% + Mancozeb 56%)
- (e) Patafol plus
- (f) Code no. BASF 474 00F (Supplied by Ceiba-Geigy)

Concentrated suspensions of these fungicides were first prepared and suitable quantities added to LBA to obtain a range of concentrations. Fifteen ml agar were used per 9 cm petri dish. Each dish was inoculated centrally with a 6 mm disc from the edge of a 1-week old colony on LBA. There were three replicate plates of each treatment and these were

incubated at 25°C in the dark. After 4 days colony growth was assessed by measuring at right angles and percentage inhibition in growth was calculated in relation to growth on unamended agar.

3.2 Screening of fungicides on nursery plants.

Four clones (PB 86, RRIC 45, RRIM 600 and RRIM 602) susceptible to bark rot and with stems of uniform size (15-25 cm circumference) were chosen. On each, at approximately 1.5 m above ground, an area of bark 30 cm long and encompassing half the circumference of the tree, was lightly scraped with a hard brush. Fungicides at concentrations given below (manufacturer's recommendations) were applied to these areas every week for 8 weeks using a paint brush.

Aliette (80% W.P.) at 1.6%

Ridomil (25% W.P.) at 0.4%

Difolatan (40% a.i.) at 1.6%

Antimucin (16.5% a.i.) at 0.8%

Oxadixyl (4% a.i.) - Sandoz 371 F4 PA09 - applied as a paste

Distilled water was used on control plants. Three trees per clone were used for each treatment. After eight applications were completed the plants were inoculated with a zoospore suspension (10^4 zoospores/ml) of P. meadii, isolate no. 362, in the following manner:

A plug of bark 2 cm diameter was removed with a cork borer at the centre of the scraped area. Exuding latex was allowed to coagulate and then removed with cotton wool. A plug (2 cm diameter) of sterilized absorbent wool, previously soaked in the zoospore suspension, was inserted into each cavity and this was covered with adhesive tape to prevent drying (Fig. 43). After 3 weeks the tape was removed and the bark pared off with a knife to expose the area infected. The lesion developed on the surface of the wood was immediately traced onto paper and the area infected was calculated using a planimeter.



Fig. 43. Method of placing inoculum in cavity made on the bark.

3.3 Effect of fungicides on the control of Phytophthora panel disease

For this experiment 258 eight-year-old mature rubber trees were selected in July 1985 within a badly infected rubber plantation showing typical symptoms of bark rot disease. An assessment of the damage shown superficially as sunken vertical depressions on the bark was made by counting these. The following fungicides were tested:

Aliette

Ridomil

Difolatan

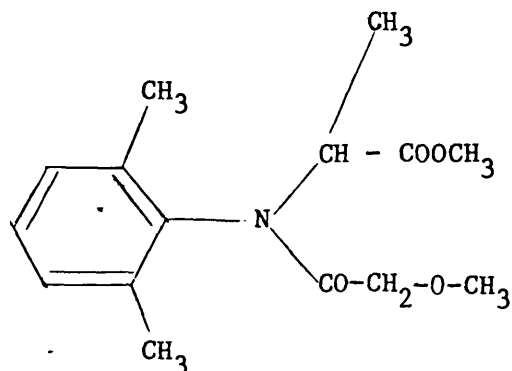
Antimucin

Oxadixyl (Sandoz 371 F4 PA09)

The concentrations used were as for the nursery plants except that a lower concentration of Difolatan (0.8% a.i.) was also included as a treatment. Forty trees were used for each treatment and 18 trees were left untreated as the control. Fungicides were applied weekly for 12 weeks to unscraped bark covering the area infected approximately 7.5 cm from the tapping cut upwards. Tapping of the rubber tree was continued as usual. Two weeks after the final application each tree was examined carefully for the number of old depressions, new depressions and their lengths. Any exudation of latex due to increased disease activity was also noted.

Metalaxyl

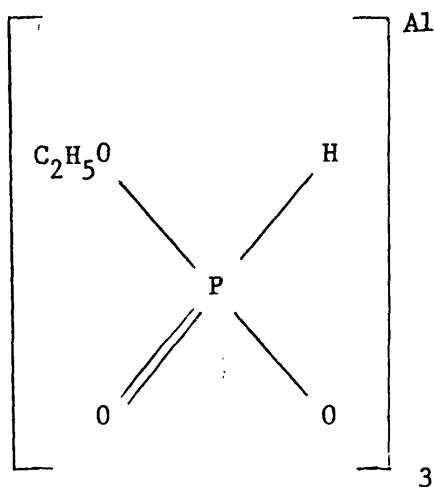
(Ridomil)



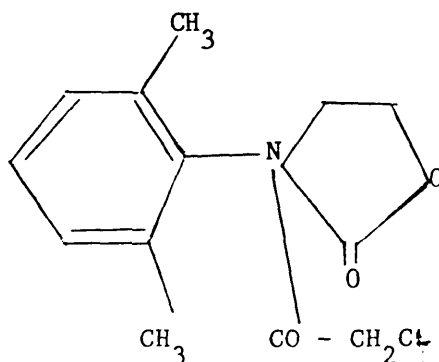
Active ingredient: N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-
alanine methyl ester

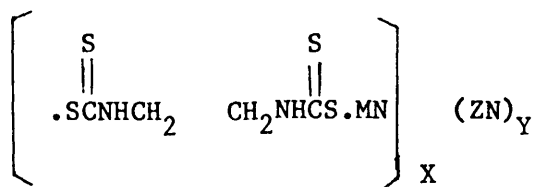
Phosethyl Aluminium

(Aliette)



Active ingredient: Aluminium tris-ethyl phosphonate

Patafol plus

Mancozeb

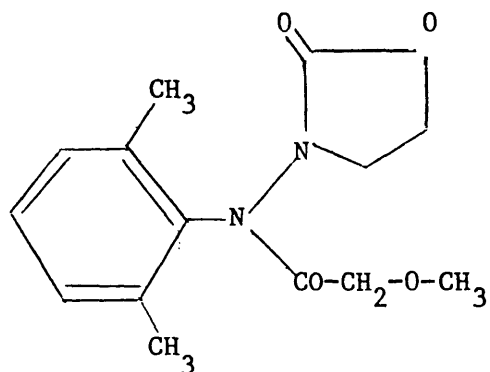
Active ingredient: [1,2-ethanedithiolbis (carbamodithioato)](2-1)]-
manganese + [1, 2-ethanedithiolbis (carbamodithioato)] (2-)] Zinc

Difolatan (captafol)

Active ingredient: CIS-N-[(1,1,2,2-tetrachloroethyl)] thio-4-
cyclohexene-1,2-dicarboximide

Oxadixyl (Sandoz 374 F₄ PA09)

Active ingredient: 2-methoxy-N-(2-oxo-1,3-oxazolidin-3-yl)
-acet-2',6'-xylidine

Antimucin

Active ingredient - Phenyl mercuric acetate

4. Results

4.1 Effect of fungicides on mycelial growth of Phytophthora species

The detailed results of the tests are given in Table 40 and the effects of the six fungicides in relation to the Phytophthora species including MF₄ are compared in Table 41.

Only the mixture of tridemorph and maneb completely inhibited growth of all seven Phytophthoras within the range of concentrations (5-200 ppm) tested. Mancozeb (at 200 ppm or less) completely inhibited all Phytophthoras except P. citrophthora. Clearly, P. citrophthora was generally the least sensitive of all those tested (Table 41).

Apart from this species, Patafol plus and Recoil also caused complete inhibition of growth of other species at concentrations 200 ppm or less, though Patafol plus was not tested against the isolate of P. palmivora from rubber. Complete inhibition by metiram + ofurace was obtained with P. citricola and the two isolates of P. palmivora but not with the remaining species.

The effects of BASF 474 00F were somewhat unusual. Some inhibition was obtained at 10 ppm with all isolates but in each instance increasing the concentration to 200 ppm resulted in relatively little increase in % inhibition. The results suggest that the solubility of this material may be very low indeed and this aspect needs to be examined in further tests.

More experiments are also needed to determine the dose of each fungicide inhibiting growth of the isolates by 50%. The data of Table 41 are only the approximate ranges based on limited evidence. Nevertheless they indicate that P. citrophthora is relatively the least sensitive to most fungicides with the possible exception of Recoil, and that P. citricola is particularly sensitive to Mancozeb and to a mixture of tridemorph and maneb. Thus, they emphasize generally the efficacy of this mixture.

A few further tests with Patofol plus on five Phytophthora isolates are summarized in Appendix III. These illustrated further the relatively insensitivity of P. citrophthora.

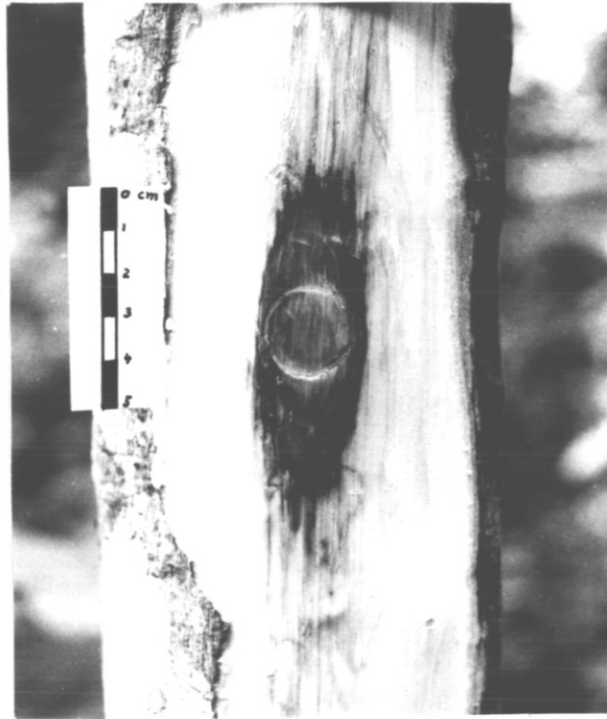
4.2 Screening of fungicides on nursery plants.

This experiment gave rather variable results with some fungicides. Nevertheless it was clear that Oxadixyl (Sandoz 374 F₄ PA09) controlled the spread of the disease significantly, in all four clones tested (Table 42 and Fig. 44b). Trees treated with Difolatan, Ridomil and Antimucin generally had smaller lesions compared to the untreated trees (Figs. 44a,c,d & e) but this trend was not consistent for all clones. Aliette did not give satisfactory results (Fig. f). With three of the four clones treated with this fungicide lesions were as large or larger than in the control (Table 42).

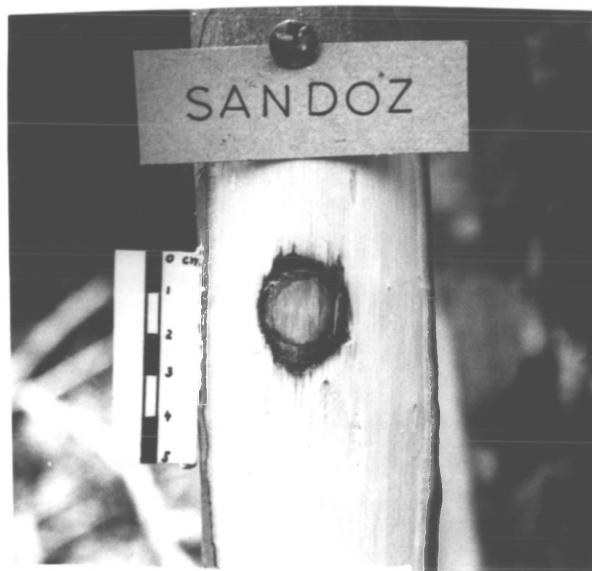
4.3 Effect of fungicides on the control of Phytophthora panel disease.

The number of depressions on the bark decreased after the application of the fungicide but increased on the untreated (control) trees. However, the total length of the depressions increased in all cases particularly so in the trees treated with Antimucin. This indicates that some depressions extended in length on the bark, even after treating with fungicides (Table 43). New depressions developed closer to the tapping cut in all treatments but the number and their length were much less in the trees treated with Oxadixyl (Sandoz 374 F₄ PA09) and Difolatan (1.6%). This suggests that these two fungicides are best because both reduce the occurrence of new depressions and Difolatan (1.6%) caused greatest reduction of existing depressions. In the untreated control, in addition to producing a large number of new depressions, exudation of latex on the panel, through cracked bark was observed.

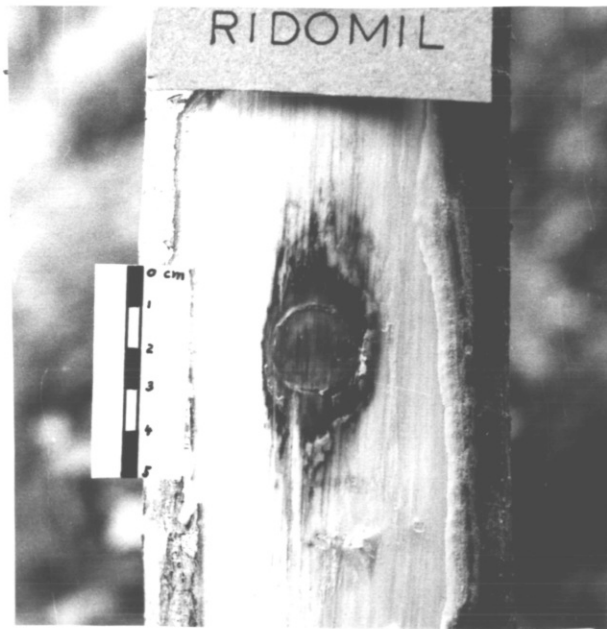
Fig. 44. Lesion development on clone PB86 in the fungicidal trial
on nursery plants (Wood exposed to show lesion)



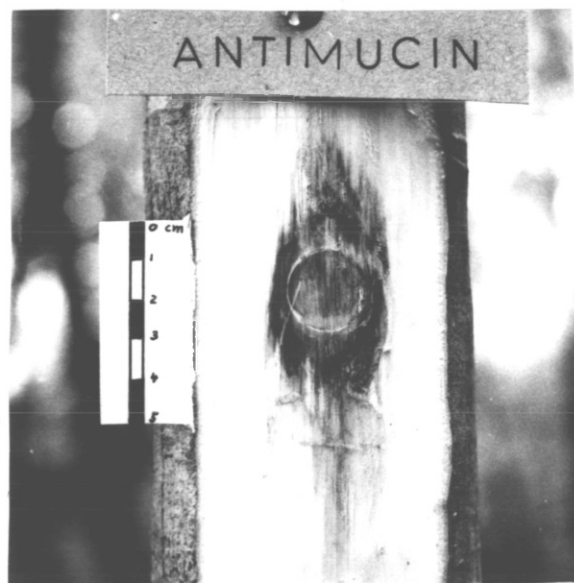
(a) Control (untreated)



(b) Oxadixyl (Sandoz - 374 F₄ PA09)



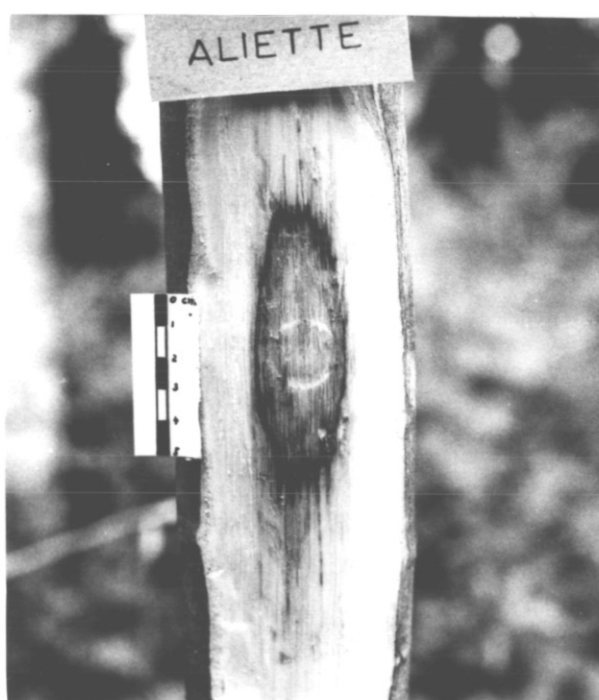
(c) Ridomil



(d) Antimucin



(e) Difolatan



(f) Aliette

Table 40 Effect of fungicides on linear growth of Phytophthora spp.

Fungicide	% Inhibition at ppm ^a of				
	5	10	50	100	200
(a) <u>P. meadii</u>					
Mancozeb	-	23	37	100	
Tridemorph + maneb	-	23	100		
Metiram + ofurace	-	19	61	82	85
BASF 474 00F	-	61	63	68	79
Patafol plus	-	25	51	83	100
Recoil	29	41	100		
(b) <u>P. palmivora</u> (isolate from rubber)					
Mancozeb	-	34	77	100	
Tridemorph + maneb	-	29	88	100	
Metiram + ofurace	-	24	86	90	100
BASF 474 00F	-	44	49	58	60
Patafol plus	-	-	-	-	-
Recoil	60	100	-	-	-
(c) <u>P. palmivora</u> (isolate from cocoa)					
Mancozeb	-	32	42	100	
Tridemorph + maneb	-	21	100		
Metiram + ofurace	-	34	82	88	100
BASF 474 00F	-	38	45	50	53
Patafol plus	-	19	58	87	100
Recoil	25	59	100		
(d) <u>P. citricola</u>					
Mancozeb	-	100			
Tridemorph + maneb	-	100			
Metiram + ofurace	-	30	81	100	
BASF 474 00F	-	50	56	60	63
Patafol plus	-	0	46	47	100
Recoil	16	45	100		
(e) <u>P. botryosa</u>					
Mancozeb	-	20	60	90	100
Tridemorph + maneb	-	24	91	100	
Metiram + ofurace	-	22	56	83	87
BASF 474 00F	-	50	56	60	68
Patafol plus	-	12	42	65	100
Recoil	24	50	1	0	
(f) MF4					
Mancozeb	-	21	39	87	100
Tridemorph + maneb	-	17	62	71	100
Metiram + ofurace	-	17	44	60	78
BASF 474 00F	-	52	56	65	68
Patafol plus	-	0	24	49	100
Recoil	9	14	51	1	0

Table 40 (continued)

Fungicide	% Inhibition at ppm ^a of				
	5	10	50	100	200
(g) <u>P. citrophthora</u>					
Mancozeb	-	10	29	45	61
Tridemorph + maneb	-	13	48	60	100
Metiram + ofurace	-	12	22	50	76
BASF 474 00F	-	44	47	49	54
Patafol plus	-	1	12	39	88
Recoil	11	18	60	61	-

^a ppm of active ingredient

Values for % inhibition are based on means of 3 replicate plates at each concentration

Table 41. Comparison of the efficacy of fungicides in reducing linear growth of Phytophthora species.

Species	Range (ppm) for 50% inhibition by					
	Mancozeb	Tridemorph + maneb	Metiram + ofurace	BASF474 00F	Patafol plus	Recoil
<u>P. meadii</u>	50-100	10-50	10-50	< 10	10-50	50-100
<u>P. palmivora</u> (R)	10-50	10-50	10-50	50-100	-	< 5
<u>P. palmivora</u> (C)	50-100	10-50	10-50	100	10-50	10-50
<u>P. citricola</u>	< 10	< 10	10-50	10	100-200	50-100
<u>P. botryosa</u>	10-50	10-50	10-50	10	50-100	10
MF ₄	50-100	10-50	50-100	<10	100-200	50-100
<u>P. citrophthora</u>	100-200	50-100	100	100-200	100-200	10-50

R - isolate from rubber

C - isolate from cocoa

Table 42. Effect of fungicides on nursery plants

Fungicide	Mean lesion area (cm ²) in clone			
	RRIC45	RRIM600	RRIM602	PB86
Aliette	13.3	14.5	12.9	17.8
Ridomil	13.9	9.8	8.0	12.3
Difolatan	11.6	13.6	12.9	12.6
Antimucin	11.1	10.8	10.4	16.5
Oxadixyl (Sandoz 371F ₄ PA09)	8.9	7.9	6.5	6.4
Untreated (control)	15.5	19.2	11.6	13.4

Variance ratio (F values): fungicides 7.67 P 0.001);

clones 2.4 (n.s); fungicides x clones 1.32 (n.s).

Table 43. Lesion development on clone PB86 in the fungicidal trial
on infected mature trees

Treatment	Mean no. depressions ^a		Mean length of depressions ^a (cm)		New depression ^a	
	Before	After	Before	After	no.	Length (cm)
Antimucin	8.7	5.6	3.3	9.2	0.9	3.4
Oxadixyl (374 F ₄ PA09)	7.2	4.0	3.7	7.3	0.3	1.9
Ridomil	7.8	4.3	3.4	6.7	1.4	3.4
Aliette	7.4	4.7	3.9	7.8	0.9	3.8
Difolatan (A)	8.7	5.5	3.4	6.9	1.3	3.9
Difolatan (B)	10.2	4.7	3.1	6.6	0.2	4.1
Untreated (control)	7.7	8.1	3.8	6.8	11.7	3.4

^a Each reading is a mean of 40 trees except in the untreated (Control) where it is a mean of 18 trees.

Discussion

The trials with the six fungicides in this investigation were limited in their scope but they indicate some features of interest. The fungicides were effective against the isolates of Phytophthora tested except in the case of P. citrophthora and MF₄ isolates which needed higher concentrations. The compound Oxadixyl seems to have a high fungitoxic activity against the Phytophthora species tested, controlling the spread of the lesions significantly on all four clones in the nursery. Its control of the panel disease in mature rubber in the field was also noteworthy in relation to its suppression of new depressions. Apart from being effective, this formulation has the added advantage of remaining on the panel for a long period of time without being washed away by rains.

Difolatan is also a promising fungicide and it should be used at a higher concentration of 1.6%. Antimucin being non-systemic could be easily washed off from the panel during rains. The increase of length of depressions in the infected trees after application of Antimucin was clearly seen in this study. This is perhaps due to the washing away of the fungicides during rains. Aliette and Ridomil though slightly better than Antimucin, is moderate in its activity against Phytophthora panel disease in rubber. Oxadixyl and Difolatan are the two promising fungicides that could be used both as a prophylactic and a curative measure against Phytophthora infection and it may be possible to use these as substitutes for currently used Antimucin. However, further tests of oxadixyl and difolatan are required especially to determine the persistence and their efficacy over several seasons. The costs of these materials also need to be appraised before final recommendations are made.

GENERAL DISCUSSION

Disease caused by Phytophthora spp., is present in all countries where rubber is grown. The causal organisms have been identified in some countries but not in others. Nor have there been any comparative studies on a range of isolates collected throughout the World.

The main features of the experiments have already been discussed in the four sections. The emphasis here therefore, is on those either of particular interest or which require further study.

From this world-wide collection of isolates from rubber, five species were distinguished: P. palmivora, P. meadii, P. botryosa, P. citricola, P. citrophthora and one currently designated as MF₄. Isolates from cocoa included two species P. palmivora and P. katsurae. The presence of P. citricola on rubber and P. katsurae on cocoa are new records. The causal organism for Phytophthora infection on rubber in Ivory Coast as P. citrophthora and the presence of the same fungus in Indonesia on rubber is recorded for the first time. Although only a few isolates were studied, these findings are noteworthy. The need to examine more isolates from some countries particularly from Ivory Coast and Indonesia is emphasized here.

The importance of pedicel length as a reliable diagnostic character amongst several morphological characters, is confirmed by this study. This has been emphasized also by many previous workers (Griffin, 1977, Tsao & Tumakate, 1977, Brasier & Griffin, 1979).

In some countries both compatibility types of a Phytophthora species are present and in some, only one type. In countries where both types are present, such as India, Thailand and Malaysia there is the possibility of oospores forming, freely, thus resulting in genetic variation. However, compatibility between different species was also

exhibited in this study, but the role of oospores in the epidemiology of the disease remains uncertain as ^{they are} often difficult to germinate.

Petch (1910) suggested that if cocoa and rubber are interplanted the Phytophthora in one host might infect the other. This aspect has not been investigated fully although cocoa has been considered as a suitable crop to plant with rubber, as it prefers some shade in the early stages of its life. As the crop needs more light in the adult tree stage, wider spacings have been worked out to provide sufficient light when grown with rubber. The present study however, clearly indicates that species pathogenic to rubber could also be pathogenic on cocoa and vice versa. Inoculations of rubber petioles and cocoa pods with isolates of rubber and cocoa showed varying degrees of susceptibility. The presence of abnormal oogonia has been observed in this study. A high rate of abortive oogonia have been previously reported by Sansome and Brasier in 1973, in mating two compatible isolates of P. infestans and selfing of an A₂ isolate of P. infestans by the "Trichoderma method" (Brasier, 1971). Even though nearly one-third of the oogonia are abnormal or incompletely fused in pairings of cocoa and rubber isolates, the presence of many normal oogonia indicates the high risk of interplanting cocoa with rubber.

Both mycelia and sporangia are not long term survival propagules of Phytophthora meadii. The fungus could be recovered from mycelium incubated in soil only for 3-4 weeks. Sporangia too are rapidly decayed in soil and most sporangia are degenerated in a short time of three days. However, some germinate and therefore, it was possible to recover the fungus upto about 3 weeks. The mycelium within the host tissue could remain viable for longer periods upto 6 weeks. The fungus also remains in infected petioles as thin-walled chlamydospores. Later they become thick-walled and remain in the petiole tissue till it is fully decayed.

It is most likely that these thick-walled chlamydospores are the means by which P. meadii perennates in soil. However, this fact has to be followed up with further experiments. For this, first techniques have to be developed to produce them in large numbers. This could be done by extracting them from infected petioles or by inducing thin-walled chlamydospores produced in culture, in suitable media to become thick-walled preferably by the use of soil extracts. Their germination then has to be assessed particularly in relation to the rubber host tissue.

Fungicides may be used either protectively to prevent the disease becoming established on the host or curatively to reduce its effect once infection has occurred. Protective action against Phytophthora panel disease (bark rot) is important especially during the rainy seasons. Leaf fall due to Phytophthora in young plantations is generally controlled by using copper fungicides formulated in oil. This increases the tenacity of the fungicide and is suitable as a low-volume application. Leaves in mature plantations are generally not protected due mainly to the inaccessibility and the increased cost in using suitable machinery. The panels are thus protected routinely during the pod and leaf fall phase of the disease. Copper fungicides however, cannot be used on the panels as rubber latex can get contaminated with copper resulting in a lower grade product. Hence suitable fungicides have to be sought from time to time. The selected fungicides must also resist weathering by wind and water and oxidation and be safe to handle and apply.

Some fungicides particularly oxadixyl and Difolatan proved to be promising. These were effective both on immature nursery plants and mature rubber trees in controlling the disease. Both preventive and curative action of the Oxadixyl fungicide was noteworthy.

Though they are effective in short-term trials, their long-term activity has to be assessed as some pathogens are known to have developed resistance to continuous use of them. In this context, the use of mixtures of fungicides and/or alternate use of different effective fungicides particularly as prophylactive applications could reduce the chances of developing resistant strains of the fungus.

As fungicides have to be used regularly on the panels during the long disease period, the cost of any selected fungicide has to be assessed and the availability has to be assured.

REFERENCES

- ALAVI, A. & STRANGE, R.N. (1979). A baiting technique for isolating Phytophthora drechsleri, causal agent of crown rot of Cucumis species in Iran. Plant Disease Reporter 63, 1084-1086.
- AL-HEDAITHY, S.A.A. & TSAO, P.H. (1979). Sporangium pedicel length in Phytophthora species and the consideration of its uniformity in determining sporangium caducity. Transactions of the British Mycological Society 72, 1-13.
- ANONYMOUS. (1979). Phytophthora botryosa Chee, causing abnormal leaf fall disease on Hevea brasiliensis in South Andaman Island in India. Rubber Digest 1, 9-10.
- ANN, P.J. & KO, W.H. (1980). Phytophthora insolita, a new species from Taiwan. Mycologia 72, 1180-1185.
- ASHBY, S.F. (1922). Oospores in culture of Phytophthora faberi. Kew Bulletin 9, 257-262.
- BANIHASHEMI, Z. & MITCHELL, J.E. (1976). Factors affecting oospore germination in Phytophthora cactorum the incitant of apple collar rot. Phytopathology 66, 443-448.
- BANIHASHEMI, Z. & MITCHELL, J.E. (1980). Photobiology of oospores of P. cactorum and P. citricola (Abstract). Proceedings of the American Phytopathological Society 14, 88.
- BLACKWELL, E. & WATERHOUSE, G. (1931). Spores and spore germination in the genus Phytophthora. Transaction of the British Mycological Society 15, 294-310.
- BRASIER, C.M. (1967). Physiology of reproduction in Phytophthora. Ph.D. thesis, University of Hull, England. 220pp.
- BRASIER, C.M. (1969). The effect of light and temperature on

- reproduction in vitro in two tropical species of Phytophthora. Transactions of the British Mycological Society 52, 105-113.
- BRASIER, C.M. (1971). Induction of sexual reproduction in single A_2 isolates of Phytophthora spp., by Trichoderma viride. Nature (London) New Biology 231, 238.
- BRASIER, C.M. (1975): Stimulation of sex organ formation in Phytophthora by antagonistic species of Trichoderma I. The effect in vitro. New Phytology 74, 183-194.
- BRASIER, C.M. & MADEIROS, A.G. (1978). The karyotype of Phytophthora palmivora. morphological form 4. Transactions of the British Mycological Society 70, 295-297.
- BRASIER, C.M. & GRIFFIN, M.J. (1979). Taxonomy of 'Phytophthora palmivora' on cocoa. Transactions of the British Mycological Society 72, 111-143.
- BRASIER, C.M., GRIFFIN, M.J. & MADDISON, A.C. (1981). The cocoa black pod Phytophthoras. In Epidemiology of Phytophthora on cocoa in Nigeria (ed. P.H. Gregory & A.C. Maddison), Phytopathological Paper No. 25, Commonwealth Mycological Institute. Kew, Surrey, England. 65pp.
- BRUCK, R.I., GOODING, Jr., G.V. & MAIN, C.E. (1981). Evidence for resistance to metalaxyl in isolates of Peronospora tabacina. Phytopathology 71, 558 (Abstr.)
- BUTLER, E.J. (1907). An account of the genus Pythium and some Chytridiaceae. Memoirs of the Department of Agriculture in India, Botanical Series 1, 82-84.
- BUTLER, E.J. (1910). The bud-rot of palms in India. Memoirs of the Department of Agriculture in India, Botanical Series 3, 221-280.
- CAMPBELL, W.A. (1949). A method of isolating Phytophthora cinnamomi directly from soil. Plant Disease Reporter 33, 134-135.

- CHAN, C.L. & LEE, B.S. (1973). A preliminary survey of cocoa diseases in West Malaysia. Malaysian Agricultural Research and Development Institute Research Bulletin 1, 22-31.
- CHEE, K.H. (1969). Variability of Phytophthora species from Hevea brasiliensis. Transactions of the British Mycological Society 52, 425-436.
- CHEE, K.H. (1973). Production, germination and survival of chlamydospores of Phytophthora palmivora from Hevea brasiliensis, Transactions of the British Mycological Society 61, 21-26.
- CHEE, K.H. & FOONG, K.M. (1968). Use of cacao pod for recovering Phytophthora species pathogenic to Hevea brasiliensis. Plant Disease Reporter 52, 5.
- CHEE, K.H. & GREENWOOD, J.M.F. (1968). Phytophthora leaf fall and pod rot of Hevea brasiliensis in Thailand. Plant Protection Bulletin of the Food and Agriculture Organization 16, 1.
- CHEE, K.H. & KAI-MING, Z. (1985). Diseases of Hevea in South Bahia, Brazil, caused by Phytophthora spp. Planter Kuala Lumpur 61, 299-305.
- CHEE, K.H., LIM, T.M. & WASTIE, R.L. (1967). An outbreak of Phytophthora leaf fall and pod rot on Hevea brasiliensis in Malaysia. Plant Disease Reporter 51, 443-446.
- CLINTON, G.P. (1911). Oospores of potato blight, Phytophthora infestans. Connecticut Agriculture Experimental Station Biennial Report 1909, 753-774.
- COOKE, L.R., CLIFFORD, D.R. & HOLGATE, M.E. (1981). Control of potato late blight (Phytophthora infestans) with systemic fungicides. Pesticide Science 12, 678-684.
- DADE, H.A. (1927). Economic significance of cacao pod diseases and

- factors determining their incidence and control. Bulletin of the Department of Agriculture, Gold Coast 6, pp. 58.
- DANCE, M.H., NEWHOOK, F.J. & COLE, J.S. (1975). Bioassay of Phytophthora spp. in soil. Plant Disease Reporter 59, 523-527.
- DANTANARAYANA, D.M., PERIES, O.S. & LIYANAGE, A. de S. (1984). Taxonomy of Phytophthora species isolated from rubber in Sri Lanka. Transactions of the British Mycological Society 82, 113-126.
- DAVIDSE, L.C., LOOIJEN, D., TURKENSTEEN, L.J. & VAN DER WAL, D. (1981). Occurrence of metalaxyl resistant strains of Phytophthora infestans in Dutch potato fields. Netherlands Journal of Plant Pathology 87, 65-68.
- de BARY, A. (1887). Comparative Morphology and Biology of the fungi, Mycetozoa and Bacteria. Oxford, Clarendon press, 522pp.
- DIJKMAN, M.J. (1951). Hevea thirty years of research in the Far East Coral Gables, Florida. University of Miami Press.
- DOUCHET, J.P., ABSI, M., HAY, S.J.B., MUNTAN, L. & VILLANI, A. (1977). European results with DPX 3217, a new fungicide for the control of grape downy mildew and potato late blight. Proceedings of the 9th British Crop Protection Conference 2, 535-540.
- DUNCAN, J.M. (1976). The use of bait plants to detect Phytophthora fragariae in soil. Transactions of the British Mycological Society 66, 85-89.
- DUNCAN, J.M. (1977). Germination in vitro of Phytophthora fragariae oospores from infected root tissue. Transaction of the British Mycological Society 69, 391-395.
- ECKERT, J.W. & TSAO, P.H. (1960). A preliminary report on the use of pimaracin in the isolation of Phytophthora species from root tissues. Plant Disease Reporter 44, 660-661.
- ECKERT, J.W. & TSAO, P.H. (1962). A selective antibiotic medium for

isolation of Phytophthora and Pythium from plant roots. Phytopathology 52, 771-777.

ENGLANDER, L., MERLINO, J.A. & MCGUIRE, J.J. (1980). Efficacy of two new systemic fungicides and ethazole for control of Phytophthora foot rot of rhododendron and spread of Phytophthora cinnamomi in propagation benches. Phytopathology, 70, 1175-1179.

ENGLANDER, L. & ROTH, L.F. (1980). Interaction of light and sterol on sporangium and chlamydospore production by Phytophthora lateralis. Phytopathology 70, 650-654.

ENGLANDER, L. & TURBITT, W. (1979). Increased chlamydospore production by P. cinnamomi using sterols and near ultra-violet light. Phytopathology 69, 813-817.

FARIAH, A., MENGE, J.A., TSAO, P.H. & OHR, H.D. (1981). Metalaxyl and elfosite aluminium for control of Phytophthora gummosis and root rot on citrus. Plant Disease 65, 654-657.

FIRMAN, I.D. (1974). Cocoa canker. In Phytophthora disease of Cocoa (ed. P.H. Gregory), pp. 131-140. London:Longman.

FORSTER, H., RIBEIRO, O.K. & ERWIN, D.C. (1981). Germination of oospores of Phytophthora megasperma f.sp. medicaginis (Abstract) Phytopathology 71, 874.

FREZZI, M.J. (1950). Las especies de 'Phytophthora' en la Argentina. Revista de investigaciones Agrícolas 4, 47-133.

GADD, C.H. (1924). Phytophthora faberi Maubl. Annals of the Botanical Gardens, Peradeniya, 9, 47-89.

GALINDO, A.J. & GALLEGLY, M.E. (1960). The nature of sexuality in Phytophthora infestans. Phytopathology 50, 123-128.

GALLEGLY, M.E. (1983). New criteria for classifying Phytophthora and critique of existing approaches. In Phytophthora. It's Biology, Taxonomy, Ecology and Pathology. (ed. D.C. Erwin, S.

- Bartnicki - Garcia & P.H. Tsao). The American Phytopathological Society, St. Paul, Minnesota, 162-172.
- GISI, U. & MEYER, D. (1973). Okologische Untersuchungen an *Phytophthora cactorum* (Leb et Cohn) Schroet im Boden mit direkten Beobachtungsmethoden. *Phytopathologische Zeitschrift* 76, 276-279. Cited by N. Malajczuk. Microbial antagonism to *Phytophthora* In: *Phytophthora Its Biology, Taxonomy, Ecology and Pathology*. Ed: D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao. The American Phytopathological Society, St. Paul, Minnesota.
- GREEN, R.J. & PRATT, R.G. (1970). Root rot of black walnut seedlings caused by *Phytophthora citricola*. Plant Disease Reporter 54, 583-585.
- GREGG, M. (1957). Germination of oospores of *Phytophthora erythroseptica*. Nature (London) 180, 150.
- GRIFFIN, M.J. (1977). Cocoa *Phytophthora* Workshop, Rothamsted Experimental Station, England, 24-26 May 1976. PANS 23, 107-110.
- HAAS, J.H. (1964). Isolation of *Phytophthora megasperma* var. *sojae* in soil dilution plates. (Abstr.). Phytopathology 54, 894.
- HARNISH, W.N. (1965). Effect of light on production of oospores and sporangia in species of *Phytophthora*. Mycologia 57, 85-90.
- HARTILL, W.F.T. (1980). Spray and seed tuber treatments for late blight control in potatoes. Plant Disease 64, 764-766.
- HICKS, P.G. (1967). Observations on the diseases and conditions of cacao pods in Papua and New Guinea - pod losses 1962-65. Papua New Guinea Agricultural Journal 19, 5-9.
- HO, H.H. (1978). Hyphal branching systems in *Phytophthora* and other phycomycetes. Mycopathologia 64, 83-86.
- HO, H.H., LIANG, Z.R., ZHUANG, W.Y. & YU, Y.N. (1984). *Phytophthora*

- spp., from rubber tree plantations in Yunnan Province of China.
Mycopathologia 86, 121-124.
- HOLDAWAY, B.F. & TSAO, P.H. (1972). Longevity of chlamydospores of Phytophthora parasitica in water. Plant Disease Reporter 56, 971-973.
- HONOUR, R.C. & TSAO, P.H. (1974). Production of oospores by Phytophthora parasitica in liquid medium. Mycologia 66, 1030-1038.
- HOTSON, J.W. & HARTGE, L. (1923). A disease of tomato caused by Phytophthora mexicana sp. nov. Phytopathology 13, 520-530.
- HUGUENIN, B. (1974). Influence des conditions de culture sur la formation et la germination in vitro des chlamydospores du Phytophthora palmivora. Ann. Phytopathol 6, 425-440.
- HUGUENIN, B. & BOCCAS, B. (1971). Role de quelques facteurs dans la formation et la germination des oospores chez le Phytophthora palmivora. Butl. Annales De Phytopathologie. 3, 353-371.
- HUGUENIN, B. & JACQUES, R. (1973). Etablissement du spectra d'action de la lumiere sur la sporogenese et l'oogenese de souches du Phytophthora palmivora Butl. Compte Rendus Hebdomadaire de Seances de L'acadmie des Sciences Series D. 276, 725-728.
- HWANG, S.C. & KO, W.H. (1978). Biology of chlamydospores, sporangia, and zoospores of Phytophthora cinnamomi in soil. Phytopathology 68, 726-731.
- KADOOKA, J.Y. & KO, W.H. (1973). Production of chlamydospores by Phytophthora palmivora in culture media. Phytopathology 63, 559-562.
- KANNAIYAN, J., RIBEIRO, O.K., ERWIN, D.C. & NENE, Y.L. (1980). Phytophthora blight of pigeon pea in India. Mycologia 72, 169-181.

- KANNWISCHER, M.E. & MITCHELL, D.J. (1978). The influence of fungicide on the epidemiology of black shank of tobacco. Phytopathology, 68, 1760-1765.
- KAOSIRI, T., ZENTMYER, G.A. & ERWIN, D.C. (1978). Stalk length as a taxonomic criterion for Phytophthora palmivora isolates from Cacao. Canadian Journal of Botany 56, 1703-1738.
- KAOSIRI, T., ZENTMYER, G.A. & ERWIN, D.C. (1980). Oospore morphology and germination in the Phytophthora palmivora complex from cacao. Mycologia 72, 888-907.
- KUAN, T.L. & ERWIN, D.C. (1980). Formae speciales differentiation of Phytophthora megaspermae isolates from soybean and alfalfa. Phytopathology 70, 333-338.
- LAZAROVITE, G., UNWIN, C.H. & WARD, E.W.B. (1980). Rapid assay for systemic fungicides against Phytophthora rot of soybeans. Plant Disease 64, 163-165.
- LEGGE, B.J. (1953). Studies on species of Phytophthora. Ph.D. Thesis, University of Birmingham. 136pp.
- LEONIAN, L.H. (1934). Identification of Phytophthora species, Bulletin of the West Virginia Agricultural Experimental Station no. 262, 35pp.
- LLOYD, J.H. (1963). The control of abnormal leaf fall disease (Phytophthora palmivora, Butler) of Hevea in Ceylon. Bulletin of the Rubber Research Institute of Ceylon, 57 67pp.
- LONG, M., KEEN, N.T., RIBEIRO, O.K., LEARY, J.V., ERWIN, D.C. & ZENTMYER, G.A. (1975). Phytophthora megasperma var. sojae: Development of wild-type strains for genetic research. Phytopathology 65, 592-597.
- MALAJCZUK, N. & THEODOROU, C. (1979). Influence of water potential on growth and cultural characteristics of Phytophthora cinnamomi.

Transactions of the British Mycological Society 72, 15-18.

- MANCO, G.R. (1974). Blight of shoots, chupons, cuttings, grafts and seedlings. In Phytophthora disease of Cocoa (ed. P.H. Gregory), pp. 149-152. London : Longman.
- MARKS, G.C. & KASSABY, F.Y. (1974). Detection of Phytophthora cinnamomi in soils. Australian Journal of Forestry 36, 198-203.
- McRAE, W. (1918). Phytophthora meadii n.sp. on Hevea brasiliensis. Memoirs of the Department of Agriculture in India, Botanical Series 9, 219-273.
- MEDEIROS, A.G. & AYRES, D.J. (1965). Ocorrência de Phytophthora palmivora (Butl.) Butl. em seringueira. CEPLAC/CEPEC Relatório Anual 1964, 31.
- MEYER, D. (1975). Zur keimung der Oosporen von Phytophthora cactorum (Leb. et Cohn) Schroet. Z. pflanzenkr. Pflanzenschutz 82, 168-172.
- MIRCETICH, S.M., ZENTMYER, G.A. & KENDRICK, J.B., Jr. (1968). Physiology of germination of chlamydospores of P. cinnamomi. Phytopathology 58, 666-671.
- MIRCETICH, S.M. & ZENTMYER, G.A. (1969). Effect of carbon and nitrogen compounds on germination of chlamydospores of Phytophthora cinnamomi in soil. Phytopathology 59, 1732-1735.
- MORTAN, H.V., STAUB, T. & YOUNG, T.R. (1978). The use of CGA 48988 for the control of Phytophthora parasitica var. nicotianae on tobacco (Abstr.). Proceedings of the 3rd International Congress on Plant Pathology, Munich, Parey, Hamburg, 383.
- NARA, J., WIDJANARKO, S. & HARTANA, I. (1968). List of the most important pests and diseases of plantation crops. Bulletin of the Balai Penelitian Perkebunan Bogor (Bogor) 2, 2.
- NEWHOOK, F.J. & JACKSON, G.V.H. (1977). Phytophthora palmivora in cocoa

- plantations in the Solomon Islands. Transactions of the British Mycological Society 69, 31-38.
- NEWHOOK, F.J., WATERHOUSE, G.M. & D.J. STAMPS. (1978). Tabular key to the species of Phytophthora de Bary. Mycological Paper no. 143, Commonwealth Mycological Institute, Kew, Surrey, England, 20pp.
- OPEKE, L.K. & GORENZ, A.M. (1974). Phytophthora pod rot : symptoms and economic importance. In Phytophthora Disease of Cocoa (ed. P.H. Gregory), pp. 117-124. London : Longman.
- ORELLANA, R.G. (1959). Variation in Phytophthora palmivora isolated from cacao and rubber. Phytophthology 49, 210-213.
- PADWICK, G.W. (1956). Losses caused by plant diseases in the colonies. Mycological Paper No. 1. Commonwealth Mycological Institute, Kew, Surrey, England. 93pp.
- PERIES, O.S. (1964). Review of the Plant Pathology Department. Annual Review of the Rubber Research Institute of Ceylon 1963, 48.
- PERIES, O.S. & DANTANARAYANA, D.M. (1966). The distribution of different sexual compatibility types of Phytophthora species affecting Hevea in Ceylon. Quarterly Journal of the Rubber Research Institute of Ceylon 42, 29-33.
- PERIES, O.S. & DANTANARAYANA, D.M. (1965). Compatibility and variation in Phytophthora cultures isolated from Hevea brasiliensis in Ceylon. Transactions of the British Mycological Society 48, 631-637.
- PERIES, O.S. & FERNANDO, T.M. (1966). Studies on the biology of Phytophthora meadii McRae. Transactions of the British Mycological Society 49, 311-326.
- PETCH, T. (1910). Cacao and Hevea Canker. Agriculture Journal of Rubber, Botanical Gardens, Peradeniya 5 (13), 143-180.
- PETCH, T. (1921). Diseases and Pests of the Rubber Tree. MacMillan,

London. 278pp.

- PETHYBRIDGE, G.H. (1914). Further observations on Phytophthora erythroseptica on the disease produced by it in the potato plant. Scientific Proceedings of the Royal Dublin Society Series 5 14, 179-198.
- PRAT, B.H., SEDGLEY, J.H., HEATHER, W.A. & SHEPHERD, C.J. (1972). Oospore production in Phytophthora cinnamomi in the presence of Trichoderma koningi. Australian Journal of Biological Science 25, 861-863.
- RAMAKRISHNAN, T.S. (1961). Experiments on the control of abnormal leaf fall of Hevea, caused by Phytophthora palmivora in South India. Proceedings of the Natural Rubber Research Conference, Kuala Lumpur 1960, 454-466.
- RANDS, R.D. (1922). Streepkanker van kaneel, veroorzaakt door Phytophthora cinnamomi n.sp. Mededelingen Instituut voor Planteziektenkundig Onderzoek Wageningen 54, 53pp.
- RAO, B., ROMANO, R., DE SOUZA, A.R. & DE CASTRO, A.M.G. (1980). Surtos de requeima de Phytophthora nas seringueiras do sul da Bahia em 1980. SUDHEVEA, 26pp.
- RIBEIRO, O.K., ZENTMYER, G.A. & ERWIN, D.C. (1976). The influence of qualitative and quantitative radiation on reproduction and spore germination of four Phytophthora species. Mycologia 68, 1162-1173.
- RICHARDSON, L.T. (1976). Basic studies on fungicides. Pesticide Research Report. 385-386.
- ROSENBAUM, J. (1917). Studies of the genus Phytophthora. Journal of Agriculture Research 8, 233-276.
- SAKATA, K. & RICKARDS, R.W. (1980). Synthesis of homothallin II. Twenty third symposium. Chemistry of natural products. Nagoya,

Japan, 165-172.

- SALVATORE, M.A., GRAY, F.A. & HINE, R.B. (1973). Enzymatically induced germination of oospores of Phytophthora megasperma. Phytopathology 63, 1083-1084.
- SANSOME, E.R., BRASIER, C.M. & GRIFFIN, M.J. (1975). Chromosome size differences in Phytophthora palmivora, a pathogen of cocoa. Nature, London 255, 704-705.
- SANSOME, E.R., BRASIER, C.M. & SANSOME, F.W. (1979). Further cytological studies on the 'L' and 'S' types of Phytophthora from cocoa. Transactions of the British Mycological Society 73, 293-302.
- SATCHUTHANANTHAVAL, V. (1963). Complementary strains of P. palmivora from Ceylon rubber. Phytopathology 53, 729.
- SATOUR, M.M. & BUTLER, E.E. (1968). Comparative morphological and physiological studies of the progenies from intraspecific matings of Phytophthora capsici. Phytopathology 58, 168-172.
- SAVAGE, E.J., CLAYTON, C.W., HUNTER, J.H., BRENNEMAN, J.A., LAVIOLA, C. & GALLEGLY, M.E. (1968). Homothallism, heterothallism and interspecific hybridization in the genus Phytophthora. Phytopathology 58, 1004-1021.
- SCHWINN, F.J. (1959). Untersuchungen Zur Systematik der Gattung Phytophthora de Bary. Archiv fur Mikrobiologie 33, 223-252.
- SCHWINN, F.J. (1979). Control of Phycomycetes : a changing scene. Proceedings of the 10th British Crop Protection Conference 3, 791-802.
- SCHWINN, F.J. (1983). New developments in chemical control of Phytophthora. In Phytophthora, Its Biology, Taxonomy, Ecology and Pathology, (Ed. D.C. Erwin, S. Bartnicki - Garcia & P.H. Tsao). American Phytopathological Society, St. Paul, Minnesota,

327-334.

- SHAW, D.S. (1967). A method of obtaining single-oospore cultures of Phytophthora cactorum using live water snails. Phytopathology 57, 454.
- SHEW, H.D., BENSON, D.M. & GRAND, L.F. (1979). A comparison of bait for isolating Phytophthora cinnamomi from soil (Abstr.) Phytophology 69, 532.
- SKIDMORE, D.I., SHATTOCK, R.C. & SHAW, D.S. (1984). Oospores in cultures of Phytophthora infestans resulting from selfing induced by the presence of P. drechsleri isolated from blighted potato foliage. Plant Pathology 33, 173-184.
- SMOOT, J.J., GOUGH, F.J., LAMEY, H.A., EICHENMULLER, J.J. & GALLEGLY, M.E. (1958). Production and germination of oospores of Phytophthora infestans. Phytopathology 48, 165-171.
- SNEH, B. & McINTOSH, D.L. (1974). Studies on the behaviour and survival of Phytophthora cactorum in soil. Canadian Journal of Botany 52, 795-802.
- TAN, A.M. (1983). A new fungicide for the control of black stripe. Planters' Bulletin, Rubber Research Institute of Malaysia, 174, 13-16.
- TAN, A.M., LEONG, M.W., JOHN, C.K. & TAN, K.J. (1977). Current status of Phytophthora diseases of rubber in peninsular Malaysia. Proceedings of the Rubber Research Institute of Malaysia Planters' Conference Kuala Lumpur 1977, 65-73.
- THANKAMMA, L., GEORGE, M.K. & GEORGE, V.K. (1968). Occurrence of two species of Phytophthora on Hevea brasiliensis in India. Rubber Board Bulletin 10, 43.
- TIMMER, L.W. (1979). Preventive and systemic activity of experimental fungicides against Phytophthora parasitica on citrus. Plant

Disease Reporter 63. 324-327.

- TSAO, P.H. (1969). Studies on the saprophytic behaviour of Phytophthora parasitica in soil. In Proceedings of the International Citrus Symposium 3 (ed. H.D. Chapman), 1221-1230. University of California, Riverside.
- TSAO, P.H. (1970). Selective media for isolation of pathogenic fungi. Annual Review of Phytopathology 8, 157-186.
- TSAO, P.H. (1971). Chlamydospore formation in sporangium-free liquid cultures of Phytophthora parasitica. Phytopathology 58, 1070.
- TSAO, P.H. & BRICKER, J.L. (1968). Germination of chlamydospores of Phytophthora parasitica in soil (Abstract). Phytopathology 58, 1070.
- TSAO, P.H., CHEW-CHIN, N. & SYAMANANDA, R. (1975). Occurrence of Phytophthora palmivora on Hevea rubber in Thailand. Plant Disease Reporter 59, 955-958.
- TSAO, P.H. & OCANA, G. (1969). Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. Nature 223, 636-638.
- TSAO, P.H., TUMMAKATE, A. & BHAVAKUL, K. (1970). Recovery of Phytophthora species from old, badly decayed, infected tissues of Hevea brasiliensis. Transactions of the British Mycological Society 66, 557-558.
- TSAO, P.H., TUMMAKATE, A. (1977). The identify of a Phytophthora species from black pepper in Thailand. Mycologia 69, 631-637.
- TUCKER, C.M. (1931). Taxonomy of the genus Phytophthora de Bary. Missouri Agricultural Experiment Station Research Bulletin 153, 208pp.
- TURNER, P.D. (1960). Strains of Phytophthora palmivora (Butl.) Butl. from Theobroma cacao L. I. Isolates from West Africa.

- Transactions of the British Mycological Society 43, 665-672.
- TURNER, P.D. (1961). Strains of Phytophthora palmivora (Butl.) Butl. from Theobroma cacao L. II. Isolates from non-African countries. Transactions of the British Mycological Society 44, 409-416.
- TURNER, P.D. (1965). Behaviour of Phytophthora palmivora in soil. Plant Disease Reporter 49, 135-137.
- URECH, P.A., SCHWINN, F. & STAUB, T. (1977). CGA 48988, a novel fungicide for the control of late blight, downy mildews and related soil-borne diseases. Proceedings of the 9th British Crop Protection Conference 2, 623-631.
- WASTIE, R.L. (1973). Influence of weather on the incidence of Phytophthora leaf fall of Hevea brasiliensis in Malaysia. Journal of the Rubber Research Institute of Malaya 23, 381-390.
- WATERHOUSE, G.M. (1963). Key to the species of Phytophthora de Bary. Mycological Paper no. 92. Commonwealth Mycological Institute, Kew, Surrey, England. 22pp.
- WATERHOUSE, G.M. (1974). Phytophthora palmivora and some related species. In Phytophthora (ed. P.H. Gregory), 51-70. London : Longman.
- WATERHOUSE, G.M., NEWHOOK, F.J. & STAMPS, D.J. (1983). Present criteria for classification of Phytophthora. In Phytophthora it's Biology, Taxonomy, Ecology and Pathology (ed. D.C. Erwin, S. Bartnicki - Garcia & P.H. Tsao), 162-172, The American Phytopathological Society, St. Paul, Minnesota.
- WESTE, G. & VITHANAGE, K. (1979). Survival of Chlamydospores of Phytophthora cinnamomi in several non-sterile, host-free forest soils and gravels at different soil water potentials. Australian Journal of Botany 27, 1-9.

- WILLIAM, D.J., BEACH, B.G.W., HORRIERE, D. & MARECHAL, G. (1977). A new systemic fungicide with activity against Phycomycete disease. Proceedings of the 9th British Crop Protection Conference 2, 565-573.
- YU, J.Y., CHANG, H.S. & KO, W.H. (1981). Factors affecting the induction of sexual reproduction in Phytophthora parasitica by P. colacasiae. Journal of General Microbiology 123, 249-252.
- ZENTMYER, G.A. (1980). Phytophthora cinnamomi and the disease it causes. Monograph 10. American Phytopathological Society, St. Paul, Minnesota 96pp.
- ZENTMYER, G.A., GILPATRICK, J.D. & THORN, W.A. (1960). Methods of isolating Phytophthora cinnamomi from soil and from host tissue (Abstr.). Phytopathology 50, 87.
- ZENTMYER, G.A. & ERWIN, D.C. (1970). Development and reproduction of Phytophthora. Phytopathology 60, 1120-1127.
- ZENTMYER, G.A., KAOSIRI, T. & IDOSU, G. (1977). Taxonomic variants in the Phytophthora palmivora complex. Transactions of the British Mycological Society 69, 329-332.
- ZENTMYER, G.A., OHR, H.D. (1978). Avocado root rot. Leaflet 2440. University of California Division of Agricultural Science, Berkeley, 15pp.
- ZENTMYER, G.A., KLURE, L.J. & POND, E.C. (1979). The influence of temperature and nutrition on formation of sexual structures by Phytophthora cinnamomi. Mycologia 71, 55-67.

Appendix I

Culture media

(a) Lima bean agar (LBA)

Difco-lima bean agar was used in experiments where growth rate, sporulation and sporangial morphology were assessed. LBA at the rate of 23g/L was used, autoclaved at 15 p.s.i. for 15 min.

(b) V8 juice agar (V8 agar)

This was prepared by adding 100ml V8 juice (Cambell's), 20g New Zealand agar to 900ml distilled water and autoclaved at 15 p.s.i. for 15 min. It was used in all experiments where compatibility of isolates were tested and also when isolates were subcultured periodically.

(c) V8 juice CaCo_3 broth (clarified V8 juice)

This medium consisted of filtered V8 juice, 100ml; filtered 2% calcium carbonate, 100ml; and de-ionized water, 800ml, autoclaved at 15 p.s.i. for 20 min. This medium was used for the production of chlamydospores.

(d) Petiole extract with CaCo_3

Green healthy, mature petioles of Hevea were selected and 100g of it was cut up into approximately 2.5cm long pieces. These were washed well in distilled water to remove latex particles and boiled in 800ml of distilled water for 30 min. Petiole extract was filtered through a double layer of muslin and made up to 900ml, with distilled water. To this 100ml of filtered 2% CaCo_3 was added and the mixture was autoclaved at 15 p.s.i. for 20 min.

(e) P_{10}VP medium (Tsao and Ocano, 1969).

Seventeen grammes dehydrated Bacto corn meal agar (Difco) dissolved in 1L distilled water was autoclaved at 15 p.s.i. for 15 min. Pimaracin, Vancomycin, penta-chloro-nitrobenzene (PCNB) at 10,200 and 100 ppm a.i. respectively, first dissolved in sterile distilled water

were incorporated into the medium before pouring plates. Freshly prepared medium was used always for selective isolation of Phytophthora spp. from soil, plant parts, in studies connected with the survival of the fungus.

Appendix II

	Isolate No.	Country	Species	Compatibility group
Group A	1015,1071,1072,1093, 1145,1200,1321,1337, 1339,1350,1397,1438, 1439,1440	Malaysia	<u>P. palmivora</u>	A ₁
	1083	Malaysia	<u>P. palmivora</u>	A ₂
	8221	China	<u>P. palmivora</u>	A ₂
	PPII	Indonesia	<u>P. palmivora</u>	A ₂
Group B	1075,1117,1223,1303, 1334	Malaysia	<u>P. botryosa</u>	A ₂
	TRT1, HYN2T	Thailand	<u>P. botryosa</u>	A ₂
	HY03	Thailand	<u>P. botryosa</u>	A ₁
Group C	1P,2P,3L,4P,4L,5P,5L 6P,7P,9P,11P,11L,12B, 13P,14P,16P,16L,17P, 18P,18L,20P,21P,21L, 22P,22L,23P,24P,25P, 25L,26L,26S,27L,28P, 29B,361,362,365,434 444,445,447,456,462	Sri Lanka	<u>P. meadii</u>	A ₁
	126	Sri Lanka	<u>P. meadii</u>	A ₂
	100,103,105,109	India	<u>P. meadii</u>	A ₁
	81	India	<u>P. meadii</u>	A ₂
Group D	23B,51B,1,2,3,4,NS1, NS2,NS4,NS5	Brazil	MF ₄	A ₁
Group E	152H,15IC,27IC,53IC	Ivory Coast	<u>P. citrophthora</u>	A ₂
	PP27	Ivory Coast	<u>P. citrophthora</u>	Unknown
	8220	China	<u>P. citrophthora</u>	A ₁
	PPII	Indonesia	<u>P. citrophthora</u>	A ₂

Appendix III. Further tests with Patafol plus

Studies were made on the following aspects: (a) Production of sporangia (b) germination of sporangia (c) germination of zoospores, of different Phytophthora species in the presence of Patafol plus.

(a) Production of sporangia.

Plates used in colony growth studies were left in continuous light at 25°C for a period of 4 days. Sporangia were counted on six mycelial plugs (6 mm) removed from each plate, closest to the inoculum plug in the centre and the number of sporangia in 18 microscopic fields was recorded.

(b) Germination of sporangia

Suspensions of sporangia 10^4 /ml were prepared from one-week-old colonies grown at 25°C in the light on V_8 agar. One ml of this suspension was mixed with an equal volume of each fungicide preparation at twice the required final concentration, in a sterile 7 ml McCartney bottle. Bottles were chilled in a refrigerator at 15°C for 20 min and then returned to 25°C to stimulate release of zoospores. After 24h at 25°C sporangia were fixed, stained with lactophenol-cotton blue and examined for direct germination or release of zoospores. Sporangia were also examined before treatment to allow for sporangia that had germinated before exposure to the fungicides.

(c) Germination of zoospores

Suspensions of zoospores (10^5 /ml) were prepared by chilling sporangia from 1-week-old colonies grown on V_8 agar. One ml of each of the fungicide preparations at twice the required final concentrations was mixed with 1 ml of zoospore suspension and incubated at 25°C.

The numbers of zoospores germinated were recorded 24 h after fixing and staining with lactophenol-cotton blue.

Results

These limited tests (Appendix III, Table A) show effects which, in

general terms are similar to those noted on mycelial growth (see Chapter 4 Section 4.1). P. citrophthora was the least sensitive isolate tested, followed by MF₄, both of which required concentrations between 100 and 200 ppm Patafol plus to reduce mycelial growth by 50%. Although at 100 ppm the growth was reduced greatly, sporangia production was seen in all species except P. meadii, with P. citrophthora forming a higher number. It was observed that Patafol plus was highly toxic, preventing germination of zoospores at 50 ppm with P. meadii, P. palmivora and P. botryosa. It also greatly inhibited the germination in P. citrophthora and MF₄ isolates at 50 ppm, and exhibited complete inhibition at 100 ppm (Appendix III, Table A). Generally, zoospore germination was more sensitive to the fungicide than release of zoospores from sporangium.

Appendix III Table A Effect of Patafol plus on production of sporangia,
zoospore release and zoospore germination

Species	% Inhibition with ppm Patafol plus		
	10	50	100
(a) Production of sporangia ^a			
<u>P. meadii</u>	46	50	100
<u>P. palmivora</u> (C)	66	80	91
<u>P. botryosa</u>	32	68	91
<u>P. citrophthora</u>	51	53	64
MF ₄	26	62	88
(b) Zoospore release ^b			
<u>P. meadii</u>	17	67	80
<u>P. palmivora</u> (C)	16	59	78
<u>P. botryosa</u>	44	63	80
<u>P. citrophthora</u>	1	14	29
MF ₄	5	25	57
(c) Zoospore germination			
<u>P. meadii</u>	59	100	100
<u>P. palmivora</u> (C)	72	100	100
<u>P. botryosa</u>	62	100	100
<u>P. citrophthora</u>	31	48	100
MF ₄	41	59	100

^a Based on counts in 18 fields at x 150

^b Based on counts of 100 sporangia

^c Based on counts of 100-150 zoospores