

Diversity and Management of *Phytophthora* in Southeast Asia

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Foreword

The genus *Phytophthora* is one of the most important plant pathogens worldwide, and many economically important crop species in Southeast Asia, such as rubber, cocoa, durian, jackfruit, papaya, taro, coconut, pepper, potato, plantation forestry, and citrus are susceptible.

Although many plant pathologists and agronomists have been aware of the economic importance of phytophthora diseases in Southeast Asia, there is a lack of information on general aspects of *Phytophthora* species in the tropics. Numerous studies have been conducted over the past few decades but the general background information is often not outlined in detail, while specific information on the occurrence and economic impact of phytophthora disease is scattered in many different publications in a range of languages. There has never been a comprehensive compilation of which species appear where, on which hosts, or what economic impact phytophthora diseases have in the region. This publication attempts to consolidate this information.

By bringing together information on the identification of phytophthora diseases based on symptoms, their occurrence, economic impact and development of integrated disease management practices, the authors of this volume provide practical information to those who seek to limit the damage caused by phytophthora diseases.

The authors have also recognised the need for a comprehensive overview of all aspects involved in the development of integrated management strategies for phytophthora diseases. The authors provide practical information, advice and background information in such a way that a reader with a basic agronomic background is able to use this information to design and implement effective integrated disease management strategies for different phytophthora diseases in different parts of the world.

The book results from a workshop held in Chiang Mai, Thailand in November 2002 with the support of ACIAR and the ATSE Crawford Fund. The workshop was the outcome of two ACIAR projects 'A survey of the presence and importance of *Phytophthora* in Southeast Asia', led by Dr André Drenth of the Cooperative Research Centre for Tropical Plant Protection, Brisbane and 'Management of *Phytophthora* diseases of durian' led by Dr David Guest of the University of Melbourne, Dr Somsiri Sangchote of Kasetsart University, Thailand and Dr Nguyen Minh Chau of the Southern Fruit Research Institute in Vietnam.

The workshop was also part of the First International Conference on Tropical and Subtropical Plant Diseases, organised by the Thai Phytopathological Society.

This publication is the latest in ACIAR's monograph series and is also available from our website at <www.aciar.gov.au>.



Peter Core
Director
Australian Centre for
International Agricultural
Research

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I Introduction

André Drenth¹ and David I. Guest²

There are about 60 species in the genus *Phytophthora*, all of them plant pathogens. *Phytophthora*, the 'plant destroyer', is one of the most destructive genera of plant pathogens in temperate and tropical regions, causing annual damages of billions of dollars.

Phytophthora diseases have been well studied in the temperate regions of the world, ever since the potato late blight epidemic in Europe in 1845–47 provided the impetus for the development of plant pathology as a scientific discipline. Throughout the wet tropics, agricultural production of a large range of crops is seriously reduced due to the wide range of *Phytophthora* pathogens causing a large number of different diseases. This chapter will explore the reasons why phytophthora diseases are so devastating in the wet tropics.

There are a number of host and pathogen factors which, together with features of their interactions, make phytophthora diseases so troublesome in the wet tropics. One of the important factors to consider is that the genus *Phytophthora* does not belong to the fungal kingdom. It is an Oomycete, closely related to diatoms, kelps and golden brown algae in the Kingdom Stramenopila (Beakes 1998). These organisms thrive in the environments found commonly in the wet tropics. There are a number of additional reasons why phytophthora diseases cause so much damage in the tropics. We have grouped these into pathogen, host, environmental and agronomic factors in Table 1.1.

All *Phytophthora* species need high humidity for sporulation and the germination of sporangiospores and zoospores to initiate infections. Frequent or seasonal heavy rainfall, and high levels of humidity, are common throughout the tropical lowlands. Tropical highlands have the added problem of

heavy mist and dew during the morning and/or late afternoon, producing free water throughout the night and providing almost daily opportunities for sporangiospores to be formed, transported and start new infections.

Another important factor in the pathogenicity of *Phytophthora* is that sporangia release motile zoospores that are attracted by chemotaxis (Carlile 1983) and electrotaxis (Morris and Gow 1993) to the roots of their host plants. The ability to seek out susceptible host tissue, coupled with zoospore motility, makes these propagules extremely efficient, even at low numbers.

Another characteristic of *Phytophthora* species, and *P. palmivora* in particular, is their ability to cause multiple diseases on the same host. In this monograph, two examples discussed in detail are cocoa and durian, and while the symptoms expressed on each host are not independent of each other, they demonstrate how numerous interactions form complex disease cycles. On cocoa, *P. palmivora* causes seedling dieback, root rot, stem canker, chupon wilt, leaf blight, cherelle wilt and black pod rot. Thus, both inoculum and susceptible host tissue are continuously available, and the disease potential is always present. These factors make *P. palmivora* an important pathogen of cocoa, and demand an integrated disease management approach.

In addition to causing multiple diseases on the same host, *P. palmivora* can also attack a wide range of different host species that are widespread and/or cultivated throughout the tropics. An appendix to this monograph tabulates *Phytophthora* pathogens and their hosts in Southeast Asia. This array of potential hosts increases the amount of inoculum and the resulting disease pressure. Furthermore, the large number of perennial host crops grown in the tropics means that susceptible host material is available all year round. Consequently, there is rarely, if ever, a break in the disease cycle. Infected plant material continuously produces large numbers of sporangia that have the ability to spread and infect new host-plant material.

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Breeding for host resistance to *Phytophthora* has given mixed results. In annual crops like soybean and potatoes, breeding started for resistance that led to the selection of specific R-genes that in some cases were quickly overcome by virulent races of the pathogen. The use of race-specific R-genes led, in some cases, to boom–bust cycles, which subsequently shifted the emphasis in resistance breeding to increasing levels of non-specific resistance, which do not completely stop infection and colonisation but slow down the rate of spread of an epidemic. Although this has led to considerable success for many annual row crops, the selection for non-specific resistance in perennial tree crops is still in its infancy, and requires a serious long-term commitment. The range of diseases caused by one *Phytophthora* pathogen further complicates breeding for non-specific resistance. Screening for resistance on leaf discs and cocoa pods may not necessarily give high levels of resistance to chupon wilt and tree cankers. Without a more complete understanding of disease cycles of the pathogen and various expressions of disease resistance in different tissue of the host plant, it is difficult to make significant steps forward by focusing on isolated aspects of phytophthora diseases.

Many agronomic practices that improve production give rise to higher levels of susceptibility, disease severity and impact in the presence of the pathogen. Flood irrigation, high levels of nitrogen fertilisers, quick-growing varieties of plants, monocultures with limited genetic diversity and high orchard density are part of modern agriculture, but these features also make these agricultural systems extremely vulnerable to phytophthora diseases. It is important to seek a broader approach in agricultural production and take account of the multitude of correlated factors in an integrated manner in order to lift production and profitability. Considering the prevalence and host range of *P. palmivora*, which can cause diseases in a large range of different host species of economic importance in the tropics, disease management efforts must move beyond controlling specific diseases on a single host and consider the whole agricultural production system. Issues like intercropping with hosts susceptible to the same *Phytophthora* pathogen need to be studied in more detail. While it may seem self-evident that interplanting susceptible hosts should increase disease severity, there are no data demonstrating that mixed farming is more vulnerable to epiphytotics caused by *Phytophthora* than are monocultures. The truth may not be so simple.

Table 1.1 Characteristics that make *Phytophthora* species so successful as pathogens in the tropics.

Environment	Pathogen	Host	Agronomic practices
<ul style="list-style-type: none"> • High rainfall • High humidity • Suitable temperature • Host plants available all year round 	<ul style="list-style-type: none"> • Spread in air and/or water • Short generation time • Rapid multiplication of inoculum • Motile zoospores • Zoospores attracted to their host by electrotaxis and chemotaxis • Chlamydospores and oospores for survival outside host • Wide host range, e.g. <i>P. palmivora</i> • Disease cycle driven by free water and high humidity 	<ul style="list-style-type: none"> • Perennial host crops – host tissue present all year round • Multiple diseases caused by the same <i>Phytophthora</i> species in different tissues of the same host • Multiple host susceptibility to same <i>Phytophthora</i> pathogen • Lack of resistance in many hosts • Abundance of insect vectors • Stem, root borers and nematodes provide entry points for infection 	<ul style="list-style-type: none"> • Over-use of and/or inappropriate irrigation • Poor drainage creates ponding • Irrigation with <i>Phytophthora</i>-infected water • Orchard established on infested soil • Susceptible planting materials • Monoculture of susceptible species • Narrow spacing of trees in orchard • No break in crop cycle • Shading practices that increase humidity • Emphasis on selection and breeding for rapid growth and high yield with little resistance • Failure of chemical control in high rainfall areas • High-nitrogen inorganic fertilisers

Due to the nature of the two ACIAR projects, the first part of this monograph focuses on the *Phytophthora* pathogens present in Southeast Asia, their hosts, general biology and economics as an output of ACIAR project PHT/1996/153 (Survey and importance of *Phytophthora* in Southeast Asia). The second part of the monograph is focused on the development of integrated disease management of *Phytophthora* on durian, 'the king of fruit', as an output of ACIAR project PHT/1995/134 (Management of *Phytophthora* diseases in durian). Some of the methods described in this monograph have been implemented and have already made a significant contribution to reducing losses due to phytophthora disease in durian. There is significant scope for further implementation of the integrated disease management practices developed — on a much larger geographic scale in durian, and similar approaches could be trialled for a number of other crops, for which a range of methods are also discussed in this monograph. Our ACIAR projects show that, for this to happen, further improvements in existing technologies, and strengthening of extension networks and training, are needed throughout Southeast Asia.

Several other crops, in addition to those described in this monograph, are under serious threat by *Phytophthora* and need a similar scientific input in an effort to reduce disease losses. We hope that this book will provide the nucleus for this effort and become a valuable resource to researchers throughout the region and beyond. We hope that by producing this monograph we will eventually help smallholders throughout the tropics reduce their losses due to phytophthora diseases.

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A young Australian boy enjoys durian and Vegemite™—symbolic of the links forged between Asia and Australia through ACIAR collaboration.

2 Economic Impact of Phytophthora Diseases in Southeast Asia

André Drenth and Barbara Sendall¹

Abstract

A number of important crops grown in Southeast Asia, such as cocoa, durian, rubber, coconut, pepper, potato and citrus, are susceptible to different species of *Phytophthora*. In this chapter, we give some background on a range of crops troubled by phytophthora and discuss the economic impact of phytophthora diseases in the region. Our assessment indicates that the economic damage on the seven crops above in the five Southeast Asian countries may be as high as 2.3 billion US dollars annually.

Introduction

Many plants grown for food and fibre suffer from a range of pest and diseases. This lowers production, increases the risk of crop failure, threatens food security and reduces the profitability of agricultural enterprises. Crop production is subject to variations in the natural environment, most notably rainfall and temperature. The complex biological and chemical interactions between the crop, mineral nutrients, and the weather give rise to considerable differences in yield and quality between seasons. The presence of diseases not only requires management inputs that reduce the profitability of crop production, but also significantly increases the risk of crop failure. The presence of diseases and pests that have the ability to significantly reduce the quality and quantity of agricultural crops is superimposed on the seasonal variability of these production factors. Thus, pests and diseases lower production, reduce product quality, increase management costs, and increase the risk of crop failure. In addition, chemical control measures may have negative collateral impacts on human health and the environment.

In order to determine the economic impact of phytophthora in Southeast Asia, the background crop production figures and an estimate of the crop

value are given for each country. Although disease losses vary enormously between different regions, seasons, different plant varieties, and under different management practices, we have tried to estimate the average crop losses experienced. The economic impact we report on is a combination of disease losses experienced on average.

In order to reduce losses due to phytophthora, disease management practices are needed. Diseases and pests can be managed in a number of ways, such as the use of resistant varieties, removal of infected plant material, pruning, tree injection, improving soil health, and application of chemicals. Each management practice imposes direct and indirect costs on the grower.

Plant pathologists need to have good tools for the assessment of disease incidence, disease severity and disease impact. These tools enable a reliable assessment of:

- the presence of the disease
- economic losses due to disease
- relative disease losses in different varieties
- field experiments comparing different disease management options
- cost-effectiveness of disease management options that improve the profitability of crop production.

Although a large range of disease assessment tools is available for our target crops, they have not been used routinely. Because of the lack of robust data, many

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previous assessments of the overall disease impact in the region have been based on educated guesses.

The aim of this chapter is to (i) establish the importance and economic value of target crops in Southeast Asia, (ii) describe the importance of phytophthora diseases on these crops, and (iii) provide an overall assessment of the economic impact of phytophthora in Southeast Asia.

Cocoa

Cocoa (cacao), *Theobroma cacao*, is native to the central and western Amazon region of South America. The Mayas, Toltecs and Aztecs cultivated cocoa more than 3000 years ago (Pereira 1992). Cocoa plants were introduced to Southeast Asia via the Philippines in the 1760s (Blaaha 1992). Cocoa is now produced by small landholders and plantations across the humid lowland tropics in Africa, Asia and the Americas (Smith et al. 1992). The major producers of cocoa are the Côte d'Ivoire (Ivory Coast), Ghana, Indonesia, Malaysia, Brazil and Papua New Guinea (Table 2.1).

Indonesia

Cocoa cultivation in Asia started in Indonesia in 1779 when the Batavian Society of Arts and Sciences offered an award to the first person to plant at least 50 cocoa trees (Blaaha 1992). Cocoa has been produced on a larger scale in East Java and North Sumatra since the 1940s, with plantings covering around 6500 ha and producing about 2000 t of dry beans annually (ICCO 1998). Production was dominated by large estates or plantations, which produced high (fine) quality cocoa. Since most of the estates were planted on Java, the exported cocoa was referred to as 'Java cocoa'. During the 1970s, the area planted to cocoa increased rapidly, and within 15 years, the number of hectares planted had tripled. Importantly, smallholder plantings increased and, by 1986, comprised 58% of the total area planted (Effendi 1992). During this period of rapid expansion, Sulawesi, Kalimantan and Sumatra joined Java as production centres (ICCO 1998).

Sulawesi, with 400,000 smallholder cocoa growers, now produces 300,000 t of dry beans per annum.

The growth has been assisted by a free economy combined with government grants to buy land, low production costs and application of management practices used in plantations in Malaysia (ICCO 1998). The current prospects for growing cocoa in Indonesia are considered good, both in terms of exports and production for local consumption. Indonesia has one of the best performances among major producing countries in terms of average yields, achieving close to 1 t/ha/year; most other producing countries have substantially lower average yields (ICCO 1998). There has been a shift in production from fine cocoa to unfermented (bulk) cocoa, due to more favourable prices for the latter. In addition, the production costs for bulk cocoa are much lower than that for fine cocoa (Effendi 1992). Pod rot and stem canker caused by *Phytophthora palmivora* often cause severe losses in Indonesia. Infestation with *P. palmivora* has been reported to be heavy in Maluku, while it is sporadically found in all provinces where cocoa is grown, especially in humid environments (Soehardjan 1992).

Malaysia

Although cocoa cultivation was first reported during the 1770s in Peninsular Malaysia, widespread cultivation of cocoa did not begin until after the Second World War. The first commercial plantings were established in the 1950s in Jerangau, Peninsular Malaysia and Sabah. The introduction of new hybrids led to a rapid expansion in cocoa cultivation. The State of Sabah is the major producer of cocoa, accounting for 70% of national production. Most of the cocoa is exported as cocoa beans, while some is processed into primary products such as cocoa butter and cocoa powder before export. The majority of the cocoa products produced in Sabah are sent to Peninsular Malaysia for processing into value-added products such as chocolate and chocolate-based products (Sabah Government 2001a).

There has been a trend away from estate production of cocoa in Malaysia over the last 20 years. In 1980,

Table 2.1 Production of cocoa in selected countries (FAO 2003).

Country	Area planted (ha)	Production (t of dry beans)	Export value (USD '000)
Indonesia	490,000	426,000	788,952
Malaysia	48,000	47,661	88,268
Philippines	12,000	6,000	11,112
Thailand	800	400	741
Vietnam	na	na	na

na = data not available.

63% of the cocoa produced was grown on estates, while in 2000, only 30% of the total crop was produced on estates; 70% was produced by small landholders. The smallholder achieves lower yields than the estates, which generally have more suitable land and greater resources. In Malaysia in 1990, the average yield for a smallholder was 610 kg/ha while the estates averaged 1100 kg/ha. Production in Malaysia peaked in 1989/90 at 243,000 t (ICCO 1998). Production has declined since 1990 however, and in 2000, Malaysia produced only 98,000 t of dry cocoa beans (FAO 2001a). Malaysia has now become an importer of cocoa beans (FME 2001a). The decline is attributed to low world prices, which caused farmers to abandon cocoa and turn to more profitable crops such as oil palm. In addition, damage due to the cocoa pod borer moth (*Conopomorpha cramerella*), labour shortages, and government incentives to grow other crops caused some growers to diversify out of cocoa. Neglect of plantations has led to pests and diseases becoming a serious problem (ICCO 1998).

Philippines

Cocoa was introduced in the Philippines in 1670, and it was the first country in Asia to plant cacao and consume chocolate drinks prepared from cocoa beans. Commercial cocoa farms were planted in the mid-1950s, and the industry expanded further in the 1960s as processing facilities were constructed. In the mid-1980s, the industry expanded further still due to investment in commercial farms and grinding and processing facilities. Southern Mindanao is the largest producing region, contributing to approximately 72% of the total production for the Philippines.

Historically, Malaysia purchased most of the cocoa beans exported, while the majority of the cocoa powder and cocoa paste were shipped to Korea. Now, the United States of America (USA) is the Philippines' major market for cocoa butter while India is the sole market for Philippine cocoa paste/cocoa cake. In 1998, the Philippines imported more than 50% of its requirement for cocoa beans, the majority coming from Indonesia (DA-AMAS 1999).

Vietnam

The Vietnamese government plans to make cocoa an important crop. Vietnam has land that is suitable to grow cocoa in the south and centre of the country and low labour costs compared with countries such as Malaysia. In the Central Highlands of Vietnam, 1500–2000 ha of cocoa will be planted annually, with the target of having planted 10,000 hectares by 2006–07. It is believed that Vietnam could be exporting cocoa by as early as 2005. Vietnam could become a significant cocoa producer in Asia by 2010 (FME 2001a).

Economic importance of phytophthora diseases in cocoa

Phytophthora spp. infect the flowers, cherelles, pods, roots, stems, and leaves of cocoa plants (Thurston 1984). Black pod caused by *Phytophthora* spp. is the most destructive disease of cocoa worldwide, causing estimated losses in production in Asia, Africa and Brazil of 450,000 t annually, worth an estimated value of USD423 million. Annual crop losses may range from 30–90% (Bowers et al. 2001). The impact of the disease varies from country to country. Black pod rot occurs in almost all cocoa-producing countries, with worldwide losses estimated at 10% (Padwick 1956). Direct crop losses of up to 90% occur in wetter areas such as Nigeria (Gregory and Maddison 1981). Pod rot and stem canker caused by *P. palmivora* often cause severe losses in Indonesia. Infestation with *P. palmivora* has been reported to be heavy in Maluku, while it is sporadically found in cocoa estates with humid environments (Soehardjan 1992). A long-term field trial over a period of 10 years at Keravat in Papua New Guinea showed a mean pod loss of 17%, with a range of 5–39% (Holderness 1992). Outbreaks of black pod disease can be so severe that cocoa plantings must be abandoned. Black pod rot is attributed to four species of *Phytophthora*: *P. palmivora*, *P. capsici*, *P. citrophthora* and *P. megakarya*. The relative impact of each of these species of *Phytophthora* varies from region to region. In Southeast Asia, *P. palmivora* seems to be the principal pathogen, while *P. megakarya* has only been found in West Africa (Brasier et al. 1981). In Africa, *P. megakarya* tends to be the principal pathogen, while in the Americas, *P. capsici* and *P. citrophthora* are the main causal agents of pod rot (Erwin and Ribeiro 1996).

Phytophthora palmivora also causes stem canker and chupon wilt of cocoa. The combination of different *Phytophthora* diseases of cocoa causes losses of 20–30% of the cocoa crop worldwide (Erwin and Ribeiro 1996). A conservative, long-term average estimate for crop losses and the cost of disease management practices is in the range of 15–20%.

Durian

The 'king of fruits', durian (*Durio zibethinus* L.), is widely cultivated in the tropics of Asia. The major producers of this fruit are Thailand, Malaysia, Indonesia, and, increasingly, Vietnam (Nanthachai 1994) (Table 2.2). Durian is indigenous to the hot equatorial rainforests of Borneo, Malaysia and Indonesia. Consequently, it prefers a hot (average maximum 33°C, average minimum 22°C) humid tropical environment with high annual rainfall of 2000–3000 mm (Lim 1998a). The fruits of the durian

tree are large, weighing between 1 and 8 kg. The fruit pulp has a rich, unique flavour but it also has a strong sulphurous aroma. The pulp of the fruit is eaten raw, cooked, frozen or dried while the seeds are used to make confectionery (Smith et al. 1992).

Durian is one of the most popular and widely eaten seasonal fruits in Southeast Asia and the fruit attracts a premium price. Production in Indonesia is mainly for domestic consumption, and Malaysia still imports a significant amount of durian in its off-season. The Philippines and Vietnam also produce durian for domestic consumption (Lim 1998b). The majority of production occurs in short seasons of two or three months, although there are two fruiting seasons in Malaysia and Indonesia because the fruit is grown in areas subject to different monsoon seasons (Lim 1998b). Production in Thailand and Malaysia is highest between June and July, while harvest peaks in Indonesia from October to February (Graef and Klotzbach 1995).

Indonesia

Indonesia exported 331 t of durian in 1993, its main market being Singapore (Graef and Klotzbach 1995). Most of the fruit is produced in Java, Sumatra, Kalimantan and Sulawesi (Lim 1998b).

Malaysia

In 1991, Malaysia was a big exporter of fresh durian, its main export market being Singapore (Graef and Klotzbach 1995). Approximately 90% of the product was exported to Singapore. However, during the off-season in Malaysia, durian is imported from Thailand. Durian is grown in Peninsular Malaysia, Sarawak and Sabah. Like Thailand, there are more than 200 varieties of durian registered, but only 20 are widely used. Durian has traditionally been produced on small orchards 0.5–1.0 ha in size, but more recently 12–120 ha commercial orchards have been established (Lim 1998b).

Philippines

Although the durian industry is rapidly expanding in the Philippines, demand continues to outweigh supply. Durian is a high-value crop with great prospects for export, owing to its late fruiting season (August–November) compared to other Southeast Asian countries. The Philippines is actively pushing to increase durian production, especially in the typhoon-free areas of Mindanao. Local consumption of durian in the Philippines is only 0.2 kg/person/year, which is only a fraction of the per person consumption in the other Southeast Asian countries (e.g. Thailand, 14 kg/person/year). There is a need to plant an additional 30 000 ha of durian to meet domestic demand if consumption rises to 2 kg/person/year (Anon. 2000).

Thailand

Almost half of the durian produced worldwide is grown in Thailand. Consequently, Thailand supplies 80% of the world export trade (Guest et al. 1998). In 1993, Thailand exported 10% of its durian export as frozen product. Its main market for fresh durian is Hong Kong, but it also exports to Malaysia, Taiwan, Canada, USA, Singapore and Indonesia (Graef and Klotzbach 1995). In 1995, the area planted to durian was approximately 128,000 ha, which accounts for 11% of the total area planted for fruit production. Most of the durian production is based on four commercial cultivars, although there are more than 200 cultivars in use. Flowers are hand-pollinated to improve fruit set and yield. The harvesting process occurs between April and September, with a constant supply between the months of May and August. This is because of the diversity of cultivars and growing regions. In 1996, durian exports amounted to about 5.5% of the total production which still amounts to USD48 million to the Thai economy (Lim 1998b).

Table 2.2 Production of durian in selected countries in Southeast Asia.

Country	Area planted (ha)	Production (t)	Value (USD '000)
Indonesia ^a	36,024	200,000 ³	780,000
Malaysia ^b	106,860	200,000	1,020,000
Philippines ^a	8000	145,000	522,000
Thailand ^a	138,024	927,200	2,686,000
Vietnam ^c	40,000	110,000	330,000

^a Figures are for 1993–94 (Nanthachai 1994).

^b Figures are for 1998 (Lim 1998b).

^c Figures are for 1998 (Chau 1998).

Vietnam

The durian industry in Vietnam is small but rapidly expanding, catering mainly for the domestic market, with some export trade with Taiwan (Chau 1998). The majority of local plantings have been established from seed, rather than from selected varieties. Durian is one of the crops targeted for improvement and expansion by the Southern Fruit Research Institute (SOFRI) (Lim 1998b). Production has increased steadily over a number of years, especially in the south-east and central highlands, and the Mekong Delta region. In the past, durian orchards were established from seedlings, but grafting onto rootstocks has become more popular. Trees are rarely pruned and flowers are not hand-pollinated as they are in Thailand. On some farms, the trees are actively water-stressed to induce off-season flowering and the farmer receives a premium price for off-season fruit.

Economic importance of *Phytophthora* diseases on durian

The high rainfall conditions under which durian are grown are conducive to the development of *Phytophthora* diseases. The most serious diseases of durian are caused by *P. palmivora*. *Phytophthora palmivora* causes seedling dieback, leaf blight, root rot, trunk cankers, and preharvest and postharvest fruit rots (Lim 1998a). Postharvest fruit rots result in 10–25% losses of durian fruits (Lim 1998b).

Patch canker caused by *P. palmivora* is considered to be a major disease of durian in Malaysia (Agrolink 2001), while fruit rot caused by the same pathogen causes losses of 30% (Chau 1998). In Sabah, *P. palmivora* and, on a few occasions, *P. nicotianae* have been reported as the causal agents of durian root rot and canker (Bong 1990).

Fruit and root rot are the most serious diseases of durian in Thailand (Pongpisutta 1998). Root rot of durian caused by *P. palmivora* was first reported in Thailand in 1966 (Phavakul and Jangsri 1969). *P. palmivora* is also responsible for many other diseases of durian in Thailand.

In Vietnam, fruit and root rot are the major diseases of durian (see Chapter 4.4). In some areas, however, damage caused by *P. palmivora* due in the form of leaf blight, patch and stem canker and fruit rot is considered to be minor (Chau 1998). Stem canker and leaf blight are more widely spread than fruit rot (van Tri 1998). The incidence and severity of *Phytophthora* diseases of durian is increasing, particularly in the Mekong Delta region, which experiences periodic waterlogging. In the Soc Trang Province of the Mekong Delta region, up to 50% of durian trees were killed by stem canker.

Since multiple diseases are caused by *P. palmivora* on durian, it is difficult to estimate the economic importance. Fruit losses due to *P. palmivora* are the easiest to assess but the influence of the tree canker on the production capacity of the durian orchard is difficult to estimate. Stem cankers can kill trees, causing loss of production over a large number of years. On average, it is estimated that disease losses and the cost of control of *P. palmivora* in durians is in the range of 20–25% of production.

Rubber

Hevea brasiliensis, para rubber, has its origins in the Amazon forests of South America, and produces latex that is used to make high-quality rubber. Rubber is a major plantation crop in Southeast Asia and supplies more than 95% of the world's natural rubber, with Malaysia, Indonesia and Thailand being major producers (Smith et al. 1992).

Natural rubber is an important agricultural commodity essential for the manufacturing of a wide range of products. The largest market for natural rubber is the tyre industry. Natural rubber is sold through a complex chain of local, national and international dealers on world markets. Production of rubber from *H. brasiliensis* makes a significant contribution to the economy of many developing countries. Over 80% of production comes from small farms, each typically 2 ha or less. Thailand is the largest producer of rubber, followed by Indonesia and then Malaysia (Table 2.3). Traditionally, natural

Table 2.3 Production of rubber in selected countries in Southeast Asia in 2000 (FAO 2001b).

Country	Area planted (ha)	Production (t)	Value (USD '000)
Indonesia	2,150,000	1,488,300	839,204
Malaysia	1,400,000	768,900	521,201
Philippines	91,474	70,000	11,756
Thailand	1,520,000	2,235,680	986,268
Vietnam	412,000	290,800	250,000

rubber was an export commodity and, until recently, processing and use was mainly in the industrialised countries. In the past few years, most of the producing countries are moving to downstream processing, converting a significant proportion of their production into manufactured products for domestic use and export.

Malaysia

Rubber is the third most important commercial crop planted in Sabah after oil palm and cocoa and is mainly grown by smallholders. Rubber sheets and latex are imported to Peninsular Malaysia for downstream processing into high-value-added rubber-based products. The government is also encouraging the cultivation of rubber for the production of rubber wood, which is used to make furniture (Sabah Government 2001b). Rubber production in Malaysia fell by approximately 20% in 2000 because many estates and smallholders continued to switch from rubber to oil palm and other products (FAO 2001b).

Indonesia

Natural rubber is one of the more important export commodities in Indonesia. This commodity provides both a source of foreign exchange and also of cash income for more than 12 million people. Rubber planters in Indonesia are predominantly smallholders (84%), and hence the quality and quantity of Indonesian rubber depends mainly on the conditions used by rubber smallholders. The two main constraints to rubber production are the traditional technology using unselected seedlings, poor soil conservation, low fertiliser input, low plant maintenance, high planting density, over-tapping, and poor soil fertility. Agricultural research institutes and the government cooperate to increase smallholder productivity by providing recommended planting materials through local farmer groups, and by developing regimes for intercropping during the period before rubber trees reach maturity. Intercrops have the dual role of providing additional income as well as providing cover to reduce soil erosion. Recommended food crops for intercropping include corn, upland rice, soybean and cowpea. Pineapple/banana and chilli are the recommended horticultural crops. Chilli has a good market in Indonesia where it is an important food ingredient. Studies have shown that both food and horticultural crops can be intercropped while rubber trees are immature, with no negative effect on rubber growth (Rosyid et al. 2001).

Philippines

The area devoted to rubber plantations is approximately 92,000 ha, more than 50% of which is in western Mindanao. Of this area, 36,000 hectares are due for replanting because the trees have reached/are near their maximum productivity. If a replanting program is not implemented, it is projected that the Philippines will be a net importer of rubber within the next 10 years. Although the potential for expansion of the industry is high, production over a 10-year period increased by an average of only 3.3% per annum, and planted area increased by only 1.4%. However, over this period, the yield increased from 1810 kg/ha in 1985 to 2170 kg/ha of raw latex. The Philippines exports about 40% of its natural rubber production, its main markets being Malaysia, China and Singapore (Anon. 2001).

Thailand

Over 90% of Thailand's natural rubber and products made from rubber are exported to overseas markets. The industry in this country is highly dependent on the world market, making it sensitive to price fluctuations in international trade, which, in turn, are influenced by the prevailing global demand for natural rubber. Strong competition from other major natural-rubber-producing countries, like Malaysia and Indonesia, and climatic conditions are also important factors that significantly affect the rubber industry in Thailand. At present, the global market situation is favourable to Thai latex producers as the global demand for natural rubber products continues to grow. Malaysia, having significantly reduced its own natural rubber production, is now importing latex concentrate from Thailand for the manufacture of rubber products (Thaitec 1998).

Vietnam

The first rubber plantation was founded in Vietnam in 1897, during the era of French colonialism. After the Vietnam War ended, the Vietnamese government aimed to re-establish Vietnam as a major exporter of natural rubber. The industry was revitalised by a USD32 million loan from the World Bank in 1996 to improve rubber latex processing technology to international standards. Most of the rubber tree plantations in Vietnam are located in the southern region of the country. In 2000, the total rubber tree plantation area in Vietnam was 412,000 ha and the average annual output of natural rubber 290,800 t. Vietnam's output of natural rubber is growing at a rate of 15% per year due to the establishment of new plantings, and young trees reaching maturity. The area planted to rubber in the year 2005 is forecast to be 700,000 ha, with plantation

ownership split equally between state and privately owned companies. Vietnam's largest rubber company is the state-owned Vietnam Rubber Corporation, which has almost 60% of the total plantation area in the country, accounting for approximately 65% of latex production. Private and provincial companies own the balance and are expected to grow dramatically in the years ahead. Collection of latex from the rubber tree begins when it reaches six years of age, and the product is harvested continuously until the tree reaches 30 years of age. Latex production by rubber trees peaks at 12 years. In Vietnam, the highest latex yield is obtained from October to December, during the months immediately following the rainy season (CBC Vietnam 1998).

Economic importance of *Phytophthora* diseases in rubber

The bark of rubber trees is regularly cut to tap the latex, and hence there are a number of important wound parasites, *Phytophthora* species being the most important (Watsie 1975). Several diseases of rubber are attributed to a number of species of *Phytophthora*, including *P. botryosa*, *P. heveae*, *P. meadii*, *P. palmivora* and *P. nicotianae*. However, *P. palmivora* and *P. meadii* are isolated most frequently as the causal agents of black stripe, patch canker, green pod rot, green twig blight, and abnormal leaf fall. Of these diseases, black stripe is the most severe disease of para rubber caused by *Phytophthora* (Erwin and Ribeiro 1996) followed by leaf fall. In wet tropical areas such as southern Thailand, leaf fall is very common and can give cause a 40% drop in yield. Black stripe is most troublesome but can be kept under control by regular management of the tapping panel. Losses due to *Phytophthora* can be high if not kept under control. The losses due to *Phytophthora* and the cost of disease control is estimated at 5–10% and has been declining recently due to the planting of more resistant rubber clones.

Coconut

Coconut (*Cocos nucifera*) are one of the most valuable plant species in the tropics, providing oil, coconut milk, fibre from the husk, palm wine, and timber for furniture and construction. It is believed that coconuts originated in Asia, with some secondary centres of origin in Central and South America. Humans have distributed coconuts throughout the tropics, and since the nuts can float, the spread has also been assisted by ocean currents.

Coconut palms are tall, unbranched trees and typically grow to 20–30 m for tall varieties, while dwarf palms only reach 10 metres. The nuts are

large, 20–30 cm in diameter, weigh up to 1 kg, and have a thick, fibrous mesocarp. The hard shell (endocarp) surrounds the seed, which contains the white, meaty endosperm that envelops the coconut water. The endosperm is high in oil and when this is dried, it is called copra. Copra contains about 60–70% oil. Coconut oil is widely used in the production of margarine, food processing, and in the production of soaps and cosmetics. The market for coconut oil has suffered in recent times because of fears the highly saturated fats are linked to increases in blood cholesterol levels. Although coconut oil contains no cholesterol, it has been largely replaced by aggressively marketed soybean and maize oils from subsidised farms in Europe and the USA.

Production of coconuts starts when the trees are 6–7 years old and may be sustained for over a century. Typical production will range from 30–70 nuts/tree/year for seedling trees but hybrids may produce more. Traditionally, the coconut tree requires little attention throughout its life span of over 50 years, and therefore it is known as a 'lazy man's crop'. Smallholders produce the majority of coconuts. Large commercial farms, however, are tended and developed for improved productivity (Agustin 2001).

The substitution of coconut oil with oil palm is another factor that is affecting the global demand for coconut oil (FME 2001b). Approximately 93% of world production of coconut occurs in the Asia-Pacific region (Table 2.4). In 1996, Indonesia supplied 26% of world production of coconut, the Philippines 23%, Thailand 5%, Vietnam 2% and Malaysia 1.5% (Food Market Exchange 2001). In the 1960s, over 1 million t of copra (dried coconut meal) was traded worldwide a year. The volume declined to about 900,000 t a year in the 1970s, further declining to an annual average of 350,000 t in the 1980s. This dramatic decline was the result of establishment of domestic copra-processing plants in response to the desire of the producing countries to obtain more value-added products. The downtrend in copra exports is likely to continue (Punchihewa and Arancon 2000).

In contrast to copra, coconut oil exports increased markedly. The world annual tonnage of coconut oil exported for 1990–1994 averaged 1.6 million t, with about 55% from the Philippines (Punchihewa and Arancon 2000). World trade in coconut oil rose 75% during the 1970s and the market further improved to an average of 1.2 million t in 1980s. Coconut oil accounts for 80% of total coconut production in the Philippines. Indonesia uses the bulk of their production internally, both as food nuts and as

coconut oil. Apart from copra and coconut oil, other exports include desiccated coconut, copra meal, cocochemicals (fatty acids, fatty alcohol, methyl ether), shell charcoal and activated carbon, fibre products, coconut cream, and coconut milk powder (Punchihewa and Arancon 2000). Coconut water is used for drinking. The white meat (copra) is processed to produce coconut milk, desiccated coconut, coconut powder, and cosmetic and pharmaceutical products (MARDI 2000).

Phytophthora diseases of coconut are important in Southeast Asia, particularly in Indonesia and the Philippines where West African-bred hybrids were widely planted in the 1980s.

Malaysia

Commercial planting of coconut started as early as 1900. In Malaysia, most of the coconuts are planted along the coastal region of Peninsular Malaysia and the states of Sabah and Sarawak. Of the 246,015 ha of coconut in Malaysia in 1993, 93% were smallholder plantings. In worldwide terms, Malaysia is a small producer of coconut and many coconut growers are opting to grow the economically more attractive oil palm (MARDI 2000).

Indonesia

Production of coconut and copra is important to the economy of Indonesia. Copra produced in Indonesia accounts for 26% of world production from 32% of the world area planted to coconut. Ninety-eight per cent of coconuts are produced by smallholders who under-plant coconut with other cash and food crops (Mady 1992). Average yields of coconut are relatively low because of the advanced age of the palms, and poor crop maintenance and disease control. The introduction of high-yielding hybrids has not improved productivity significantly, despite government support schemes (Darwis 1992). Coconut is frequently planted as a shade tree for cocoa plants (Lolong et al. 1998).

Philippines

One-third of the country's arable agricultural land (which amounts to 3.31 million ha) is planted to coconut. At present, there are more than 300 million coconut trees, bearing an annual average of 12 billion nuts. In the last five years, the average production has been 2.3 million t. The Philippines supplies 64% of global coconut oil requirements. Coconut is a major source of foreign exchange – the Philippine coconut exports accounting for some 65% of the world traded coconut products. Exports earn an average of USD800 million a year. It is the top export earner on a net basis given that its raw materials and labour components are domestically based, unlike other export products. One-third of the Philippine population (approximately 24 million people) directly or indirectly benefit from the coconut industry.

The productivity of Philippine coconut plantation per hectare per year is one tonne, compared with a potential of 2–4 t/ha/year. The poor productivity is due to a lack of agricultural inputs, limited access to credit, lack of irrigation facilities, inadequate transport and roads, poor postharvest and processing facilities, the indiscriminate removal of productive trees, and the conversion of coconut lands to other commercial and agricultural enterprises. In addition, the average gross annual income of a coconut farmer is below the poverty line. Intercropping with corn, legumes, root crops or fruit trees is not widely practised, and thus the income of growers remains poor (Philippine Department of Agriculture 1999).

Thailand

Thailand is only a small producer of coconut on a worldwide basis. During 1995, most coconut produced was consumed domestically. Its main exports of coconut products are shelled coconut, coconut oil and desiccated coconut. Exports of coconut products peaked during 1995 because production of the two biggest producers, the Philippines and Indonesia, declined (FME 2001b).

Table 2.4 Production of coconut in selected countries in Southeast Asia in 2000 (FAO 2001a).

Country	Area harvested (ha)	Production (t)	Value (USD '000)
Indonesia	2,800,000	2,342,000	140,069
Malaysia	180,000	683,000	2789
Philippines ^a	3,076,647	5,761,000	686,000
Thailand	333,000	1,373,162	2870
Vietnam	161,900	939,900	1100

^a The Philippines also produced 57,610 t of coconut seed in 2000.

Vietnam

Like Thailand, Vietnam is only a small exporter of coconut.

Economic importance of *Phytophthora* disease in coconut

Rots caused by *Phytophthora* spp. lead to palm death (by bud rot) and/or yield reduction (by premature nut fall) (Waller and Holderness 1997). While most of the coconut-growing regions of the world are affected by *Phytophthora* rots, Indonesia and the Philippines are the worst affected due to the introduction of very sensitive MAWA hybrids developed in West Africa (see Chapter 6.3) (Renard 1992). In Malaysia (Sarawak), Indonesia and the Philippines, *P. palmivora* seems to be the main causal agent of disease (Blaha et al. 1994). Coconut bud rot has an irregular distribution in the field, but the highest incidence seems to correlate with the wettest areas (Waller and Holderness 1997).

Phytophthora diseases were not a major problem in the tall coconut varieties grown in Southeast Asia, causing disease losses of 5–10% (Brahmana et al. 1992). *Phytophthora palmivora* was first reported in the Philippines on coconut in 1919 as *P. faberi* (Reinking 1923). In the 1980s, 500,000 ha of land were replanted with a MAWA coconut hybrid in order to replace old and non-productive trees. This hybrid proved to be highly susceptible to *P. palmivora*. As a result, bud rot infections led to the death of thousands of palms (Concibido-Manohar and Abad 1992). Chapter 6.3 provides the full details of this disastrous germplasm-introduction program, which gave rise to financial hardship to all who planted these hybrids as they succumbed to *Phytophthora* bud rot.

Bud rot and nut fall were first reported in Indonesia in 1985, the causal agents being identified as *P. palmivora* and *P. nicotianae* (Bennett et al. 1986). During this time, outbreaks of the disease resulted in severe damage to plantations planted with MAWA germplasm (Renard 1992). Since that time, almost all areas planted with MAWA coconut in Indonesia have suffered serious damage from bud rot, with losses in excess of 80% (Darwis 1992). In some areas, stand losses of 43% can occur due to bud rot. Premature nut fall, which is the more common disease, affects nuts of 3–7 months old (Lolong et al. 1998), and can cause losses of 50–75% (Brahmana et al. 1992). The incidence of bud rot is higher in the lowland areas of Indonesia, which are poorly drained, compared to the highland areas. Resistance among coconut varieties to infection and damage by *Phytophthora* varies with location, and therefore it is recommended that several varieties be planted to

minimise damage caused by the pathogen (Mangindaan et al. 1992).

In Indonesia, although *P. palmivora* seems to be the main causal agent of bud rot and nut fall in coconut (Blaha et al. 1994; Waller and Holderness 1997), *P. arecae* and *P. nicotianae* have also been found in association with these diseases (Thevenin 1994). *Phytophthora nicotianae* is rarely encountered, and it is usually associated with cocoa and infested soil (Waroka and Thevenin 1992). Bud rot and premature nut fall are the major disease problems affecting coconut in Indonesia (Lolong et al. 1998). The highest incidence of bud rot generally corresponds to the wettest areas.

Due to the high level of susceptibility of these hybrids to bud rot they are no longer planted. Breeding and selection programs aim to produce high-yielding varieties with good levels of resistance.

This example can be used as a timely reminder that large-scale planting of highly susceptible plant material can have drastic economic consequences. A conservative estimate of the economic impact of *Phytophthora* on coconuts is 0–5%, while 10–15% losses occurred in Indonesia and the Philippines due to the large-scale plantings of the MAWA hybrid.

Pepper

Black pepper (*Piper nigrum* L.) is a member of the tropical family Piperaceae, and it is believed to be indigenous to the state of Kerala in south-western India. It is a perennial woody climbing vine with three central climbing stems and lateral stems which bear inflorescences that produce pepper berries (Holliday and Mowat 1963). Propagation of *P. nigrum* is vegetative because seedlings take longer to bear fruit than cuttings and produce highly variable dioecious progeny. The three main climbing stems are pruned frequently to stimulate the growth of lateral fruiting branches. Fruit production begins within two years of planting, and the vines can produce fruit for 12–15 years. The flower spikes are harvested at regular intervals over a 2–3 month period (Purseglove et al. 1981). Cuttings are planted in a mound of soil in which a post made from termite-resistant wood is inserted. Alternatively, concrete posts, cut-off shade trees, or brick towers may be used to support the vines. As the vines grow, they are trained around the post. There are several different types of pepper, all derived from the berries produced by *P. nigrum*.

Black pepper is prepared by drying the mature, still-green berries in the sun for 3–4 days. White pepper is prepared from fully ripened berries that are yellow to

red in colour. The pericarp is removed from the berries by soaking in water for approximately two weeks (PMB 2001). Green pepper is prepared from unripe, green berries. The berries are artificially dried, or preserved in brine, vinegar or citric acid (IPC 1999). Long pepper is derived from *P. longum*, and is not consumed on a large scale in Western society (Purseglove et al. 1981). It is, however, used widely in India (Katzer 2000). Black pepper is regarded as the world's most important spice in terms of its use and trade value (Thurston 1984). Trade in black pepper has been known since 400–300 BC (Holliday and Mowat 1963), being described by the philosopher/botanist Theophrastus (Purseglove et al. 1981).

Pepper is known as the 'king of spices', dominating 34% of the world spice trade in volume. The demand for pepper increases by about 2.5% annually, and more than 60% of pepper is used by the food industry. Prices vary substantially because of fluctuations in supply (IPC 1999). Pepper requires heavy and well-distributed rainfall and high temperatures for optimum productivity. The International Pepper Community (IPC) comprises Indonesia, Malaysia, Thailand, Sri Lanka, India and Brazil. The IPC accounts for more than 80% of the world production and export of pepper (Table 2.5). If Vietnam joins the organisation, the IPC will control 95% of world production and export (IPC 1999). Many pepper-producing countries are developing value-added pepper products for export (PMB 2001).

Table 2.5 Production of pepper^a in selected countries in Southeast Asia (FAO 2000).

Country	Area planted (ha)	Production (t)	Export value (USD '000)
Indonesia	80,000	52,188	191,241
Malaysia ^b	12,000	21,000	106,783
Philippines	na	na	224
Thailand	2500	7000	3082
Vietnam	15,000	34,000	103,000

^a Figures include white, long and black pepper.

^b Data provided by Board (2001).

Note: na = data not available; no reference has been found to pepper production in the Philippines.

Malaysia

The British organised plantings of pepper in Malaysia early in the 19th century (Purseglove et al. 1981). Malaysia is now the fourth largest producer of black pepper in the world (PMB 2001). Currently, 95% of the pepper produced in Malaysia is grown in Sarawak (PMB 2001).

Indonesia

Hindu colonists probably took pepper to Java between 100 BC and AD 600, and thus it has a long history of cultivation in Indonesia (Purseglove et al. 1981). Black pepper is considered to be one of the oldest export commodities of Indonesia (Sitepu 1993). Until the Second World War, when supply was cut off by the Japanese invasion, Indonesia was the largest supplier of black pepper in the world (Purseglove et al. 1981). It is now the second largest producer after India (PMB 2001). Mainly small landholders produce pepper and approximately 600,000 people depend upon this commodity for their livelihood (Sitepu 1993; Wahid and Zaubin 1993). Foot rot of black pepper was first recorded in Indonesia in 1936 (Muller 1936), and since then has caused large economic losses (Tsao et al. 1985).

Vietnam

Vietnam increased its production and export of pepper four-fold over a 10-year period, increasing from 8000 t in 1990 to 34,000 t in 2000 (PMB 2001). It is now the world's second-largest pepper exporter. The price for Vietnamese pepper is usually 10–20% lower than the price offered by other pepper-exporting countries. This is due to a combination of poor quality and poor marketing (Nhan Dan 2001).

Economic importance of Phytophthora diseases in pepper

Phytophthora capsici causes foot rot of black pepper. This disease is also referred to as 'sudden wilt'. An epidemic of the disease in Sarawak in the mid-1950s caused crop losses of almost 100% (Holliday and Mowat 1963), while crop losses of 40–50% due to foot rot have been recorded in other areas (Erwin and Ribeiro 1996). Foot rot is clearly the most important and destructive fungal disease of black pepper, occurring wherever the crop is grown (Holliday 1980). The disease was originally attributed to *P. palmivora* (Muller 1936; Holliday and Mowat 1963), although a number of studies on the disease recognised that the isolates from black pepper were morphologically distinct from *P. palmivora* isolates from other hosts (Holliday 1980), being grouped with *P. palmivora* MF4 (morphological form 4) types (Tsao et al. 1985). After extensive morphological and molecular studies (Tsao and Alizadeh 1988; Tsao 1991; Mchau and Coffey 1995), the causal agent of foot rot was determined to be *P. capsici* and not *P. palmivora*. *Piper betle* L. (betle vine), the leaves of which are used as a masticatory in Asia, is also attacked by *P. capsici* (Holliday and Mowat 1963).

Foot rot caused by *P. capsici* in pepper has been reported to cause an estimated annual loss of 5–10%

in Malaysia (Kueh 1979). This would be average for a situation where the disease is managed and kept under control. Thus, in Indonesia and Malaysia, a significant amount of management is applied and experience exists to control foot rot. In other countries, the disease losses are higher (10–15%), while in Vietnam they are higher still (15–20%) due to inexperience in managing foot rot, high ground water tables in some areas, and the use of susceptible varieties.

Citrus

The genus *Citrus* contains a large number of species that provide a diversity of fruits and uses. In addition, there are many species hybrids, such as the Citrange, Citrumelo and Tangelo. Most species of citrus are cultivated for fresh fruits and to make fruit juices, jams or confectionaries. All commercially important citrus fruits have originated from species native to Southeast Asia. Citrus trees and shrubs occur naturally throughout the region, and selections are widely cultivated (Table 2.6). However, little is known about the domestication process but it most likely started a long time ago since citrus already were taken from Southeast Asia for growing in the Mediterranean during the great Greek civilisation. Many other species were established in the Mediterranean during the middle ages.

Table 2.6 Production of citrus in selected countries in Southeast Asia (FAO 2000).

Country	Area planted (ha)	Production (t)	Value (USD '000)
Indonesia	100,000	680,000	260,000
Malaysia	5220	28,500	11,000
Philippines	34,674	177,266	67,000
Thailand	91,400	1,079,500	413,000
Vietnam	71,300	450,200	172,000

There are up to twelve different species of *Phytophthora* reported to cause diseases of citrus (see Table 17.1 in Erwin and Ribeiro 1996). However, the most common species causing *Phytophthora* disease in citrus in the tropics are *P. nicotianae*, *P. palmivora*, *P. citricola* and *P. citrophthora*.

Phytophthora nicotianae may be considered as the main pathogen of citrus and causes root rot, foot rot and gummosis, although it seldom causes problems higher up in the canopy. *P. nicotianae* produces abundant chlamydospores that enhance its survival in the soil. *P. citrophthora* is another important *Phytophthora* species that causes root rot, foot rot, and gummosis but also causes brown rot in citrus. *P. citrophthora* isolates do not produce

chlamydospores. *P. palmivora* can also cause severe brown rot on the fruit under wet conditions. *P. citricola* has mainly been reported as causing brown fruit rot in citrus.

Phytophthora species attacking citrus are present in the soil. Infection occurs under wet conditions when the *Phytophthora* species are induced to produce zoospores. This typically happens during prolonged periods of wet weather, especially when flooding occurs. This typically leads to infection of the roots. However, the damage due to the root rot often shows up late during the dry season when the diseased root system is unable to keep up the supply of water and nutrients.

Economic importance of *Phytophthora* diseases in citrus

Phytophthora diseases are economically important in most citrus-growing regions. Due to the number of *Phytophthora* species and the number of different diseases involved, the economic impact is difficult to estimate. In addition, the relationship between root rot and yield loss are not proportional. Losses due to *Phytophthora* vary a lot with seasonal and climatic conditions. This is especially true for brown rot, which can lead to serious losses under wet conditions while virtually absent in years with below-average levels of rainfall. Over all different citrus species, the yield losses in the USA were estimated to range from 3–6% a year. In the wet tropical areas of Southeast Asia, the yield loss is estimated to be 6–12% a year as weather conditions are more favourable for disease, and the trees are more stressed due to the presence of other diseases and extensive monsoonal wet periods.

Potato

The common potato, *Solanum tuberosum*, is a member of the large and important family Solanaceae that includes eggplant and tomato. Europeans first saw the potato in 1537 when the Spanish landed in what is now called Colombia, South America. The potato was brought back to Europe around 1570, and it was cultivated throughout the continent before 1600, and in Ireland by 1663. The cultivated potato was first introduced into North America in 1621. Potatoes are the leading starchy root crop of the subtropical countries, and one of the eight leading staple food crops of the world. Annual production of potatoes is approximately twice that of all other edible root crops combined (Ozero 1984).

The potato is becoming increasingly important in Asia and, although rice is synonymous with food in

most of Asia, almost one-quarter of the world's potatoes are now grown in Asia (Table 2.7). The potato is a short-duration crop that produces a large amount of calories in a short period of time. In addition, potato fits well into the cereal-based cropping systems found throughout Asia. The introduction of improved, short-duration varieties of rice have provided a niche for the potato crop in the agricultural production calendar (van der Zaag 1983), but unlike cereals, the potato crop does not need to grow to full maturity before harvest. The introduction of better-adapted varieties, inorganic fertilisers, fungicides and pesticides has significantly improved productivity per unit area. Improvements in transportation and postharvest handling have reduced losses, increased marketable yields and reduced marketing costs (Horton et al. 1987). However, the main reason for the expansion of potato production in Asia has been the desire by farmers to satisfy expanding markets and changing consumer preferences. Population growth and urbanisation has expanded the market for food crops and rising per capita income has stimulated the demand for more exotic foods to diversify diets. Probably the most significant factor influencing potato consumption in Asia is the growth in the fast-food industry. Over the last three decades, potato production in Asia has tripled to exceed 60 million t.

Table 2.7 Production of potato in selected countries in Southeast Asia in 1999 (FAO 2000).

Country	Area harvested (ha)	Production (t)	Value (USD '000)
Indonesia	62,776	924,058	1,892,000
Malaysia	na	na	311,000
Philippines	5216	63,520	12,704
Thailand	900	7000	38,000
Vietnam	28,022	315,950	63,190

Note: na = data not available.

However, while productivity has increased from an average of 5.9 t/ha (1961) to 13.3 t/ha over a 30-year period, the average yield of potato crops in Asia remains low in contrast to yields of approximately 39 t/ha in temperate areas. It is considered unlikely that similar yields to those achieved in the temperate zones can be realised in Asia, and a major constraint to potato production in Asia is the inadequate supply of reasonably priced, good-quality seed tubers of the desired varieties. Substantial gains in productivity can be achieved by promoting the production and use of certified seed to reduce the risk of distributing tuber-borne pathogens. Degeneration of locally sourced planting material is

rapid due to infections with several important viral diseases. Australia produces seed potatoes under a certification system and supplies the seed to Asia. Trials in Vietnam and the Philippines with Australian-produced seed potato have resulted in significant increases in productivity (Batt 1999b).

The main biotic constraints for potato production are late blight caused by *Phytophthora infestans*, bacterial wilt disease, viruses, and potato tuber moth. Researchers estimate that developing-country farmers spend \$700 million annually to control such pests. The susceptibility of potato to these pests and diseases makes the crop the number-two user of agricultural pesticides worldwide, following cotton. Results of breeding work at the International Potato Centre (CIP, Lima, Peru) with South American potatoes is aimed at developing resistance to *P. infestans* (CGIAR 2001).

Indonesia

The Dutch introduced the potato to West Java in Indonesia around 1794. By 1811, the crop was found on other Indonesian islands such as Sumatra. However, due to the warm climate, the potato never became a food of general consumption compared to the yam, arum, and sweet potato. The area planted to potato increased steadily to over 67,000 ha in 1995–97. Yields per hectare have also increased from around 6 t/ha in the early 1970s to 11.5 t/ha in 1985. Java accounts for 65% of national production, Sumatra for 10%, while the rest occurs mainly in southern Sulawesi. The potato, along with cabbage and tomato, is an important cash crop in certain highland areas where they are produced on non-irrigated land and compete with forestry. Land temporarily cleared from trees is sometimes planted with potatoes and other vegetables. Rotations found in irrigated areas include rice–potato–cabbage, rice–potato–maize, and cabbage–potato–cabbage. Rotations found in non-irrigated areas include potato–cabbage–maize and potato–maize–fallow. Many potato pests and diseases are found in Indonesia, late blight being the most important. Small farmers cultivating less than 0.5 ha often have limited access to capital – they will tend to keep their own seed tubers which are small in size and produce low yields. More affluent farmers will plant seed potatoes imported mainly from the Netherlands and Australia. Little information is available on storage of potatoes in Indonesia, but storage periods are fairly short and losses are high. Although a small portion of the annual production is exported to Singapore and Malaysia, most Indonesian potatoes are consumed domestically. Rice remains the basic staple for the general

population, supplemented by varying amounts of maize, cassava, sago and sweet potato. In Indonesia, potato is an expensive vegetable consumed only on special occasions (CIP 1988).

Malaysia

The British introduced the potato into Malaysia in the 1930s. As in the rest of Southeast Asia, the potato was a minor vegetable in production systems and was consumed largely by the non-Asian populations. Due to unfavourable growing areas, lack of seed sources, and severe late-blight problems, the area of production of potato has not increased significantly over the past few years. However, there has been a growing demand for potato, largely met through imports, due to continuing economic prosperity. The area planted to potato also increased because of government incentives, but was limited by continued late-blight attacks in the 1980s. Potatoes are produced in Malaysia in two main highland areas on Peninsular Malaysia and Sabah, where temperatures are cool (average monthly maximum 23°C, minimum 15°C). They are typical vegetable-producing areas that cater mainly to urban markets. Potatoes are cultivated in rotation with other vegetables, especially cabbage, cauliflower, tomatoes and onions. The terrain is steep and sloping, and consequently the crops are planted on terraces. Potatoes can be cultivated throughout the year in Malaysia. Approximately 30% of the potato crop produced on Sabah is exported to Singapore. Raw potato imports into Malaysia come primarily from China, the Netherlands, Taiwan and Indonesia (van der Zaag 1983).

Philippines

Although the Spanish most likely brought the potato to the Philippines, the precise date or circumstances of the introduction are unknown. However, the potato is believed to have been present in the Philippines by the late 18th century. By the late 1930s, potatoes were being produced in large quantities. Today, the potato is a high-priority crop because of its high potential yield and nutritional qualities. Potatoes generate higher returns per hectare than most other food crops. The potato has been selected by the government as one of three national priority crops for commercial development (Batt 1999a). More than 90% of the production of potatoes takes place in the highland areas of northern Luzon, followed by upland production areas of Mindanao. Scattered, but very limited, production is found in the mountainous areas of the Visayas. Almost 90% of production occurs at altitudes between 1600 and 2400 m. Domestically consumed potatoes are purchased

primarily as a luxury vegetable or a snack food (Batt 1999a; SHEL 2001).

Thailand

Potatoes were introduced during the late 19th century to the tribes of northern Thailand either from Burma (now Myanmar) or China. The potato crop gained greater attention from both growers and the government in 1955 after the successful introduction of the variety Bintje from the Netherlands. Bintje and subsequent imported varieties have replaced most local varieties. Since the late 1970s, potato-growing has been stimulated by international agencies seeking alternatives to opium poppy as a cash crop. The growth of the tourist trade and hotel industry in Thailand has led to an increase in demand for potatoes and should stimulate further interest in the crop. Potatoes are grown mainly in the mountainous regions of northern Thailand. The two production zones include the highlands where potatoes are grown all year round but primarily in the wet season; and the lower-lying valleys where potatoes are grown on flat paddy areas after rice has been harvested. In the valleys, production occurs during the cool, dry season. Hence, production takes place virtually all year long in northern Thailand. The average mean temperature during the main growing season in the northern and north-eastern highlands is around 15–20°C, with high average annual rainfall. In the lowland valley zone, potatoes are grown on irrigated, flat paddy land using imported seed, chemical fertilisers and pesticides. They are produced by specialised potato producers who are close to the major markets and have greater opportunity to get technical advice from government extension officers. Hill tribe farmers are geographically isolated from markets and grow potatoes on rain-fed slopes, using few inputs, and locally obtained seed. They are often engaged in off-farm labour activities and are relatively isolated from extension efforts due to language and cultural barriers. The vast majority of growers of any type do not cultivate more than 1 or 2 ha/year, often at different times of the season, and hence the amount of land in potatoes at any one period may be less than 0.25 ha. A few large growers have between 5 and 10 ha of land. The vast majority of farmers sell their potatoes immediately or soon after harvesting because they lack storage facilities and need rapid cash returns. Producers eat few, if any, potatoes. Thirty per cent of total potato production is consumed locally and the rest is transported to other provinces and neighbouring countries. The government does not support producer prices, and hence potato prices are governed by the market and

by seasonal variations in supply (Rhoades et al. 1988a).

Vietnam

Although the first published reference to potatoes in Vietnam was made in 1807, it is claimed that European missionaries introduced the potato to the Red River Delta in 1890. The potato remained a minor vegetable in Vietnam until the 1970s when population growth and annual typhoon damage of the rice crop motivated the government and farmers to use the dry season from November to February for potato production. Potatoes now rank third in importance after rice and maize.

A national potato program was established in 1981. Most of the potatoes in Vietnam are produced in the lowlands of the Red River Delta, being planted after rice. The use of high-yielding, early-maturing rice varieties make it possible to harvest two rice crops within 8 months, leaving 4 months in the winter for potato production. Some production occurs in the highlands of Dalat. The temperature in the main production areas fluctuates from 17°C to 26°C. The crop is allowed only a short growing time, frequently resulting in premature harvest. The main production unit in the Red River Delta is the agricultural cooperative: 30% of the crop is sold, the cooperative members consume 35%, and 30% is stored for seed for up to 9 months. Virus infection is high in Vietnam and seed degeneration is rapid. Seed potatoes are stored by farmers in their homes in areas characterised by darkness and high temperatures, usually above 25°C, and sometimes as high as 32–35°C. The storage period can be as long as 8 months, which results in decay and losses as high as 45–60%. Hanoi and Ho Chi Minh City are the main consumption centres for potatoes (Rhoades et al. 1988b).

Economic importance of Phytophthora diseases in potato

Phytophthora infestans causes late blight of potato, and leaf, stem and fruit blights of many solanaceous hosts including tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena* L.) (Erwin and Ribeiro 1996). Late blight is listed as a major disease of potato in Malaysia, Indonesia, the Philippines, Thailand and Vietnam van der Zaag 1983; CIP 1988; Rhoades et al. 1988a,b; SHEL 2001).

Late blight is the most important disease of potatoes in Indonesia, the Philippines, in the hills of northern Thailand, and Vietnam (CIP 1988; Rhoades 1988a,b; SHEL 2001). Postharvest rot of tubers caused by *P. infestans* is also a significant problem. In Malaysia, late blight is a problem during the rainy months

from April to November. As a result, the main growing period is from December to April when less rainfall occurs (van der Zaag 1983).

All these reports indicate that late blight caused by *P. infestans* is the most important potato disease in the Southeast Asian region. Losses vary enormously between regions, varieties, and the wet and dry seasons. Potatoes are frequently sprayed with protectant fungicides to prevent infection. This intense management comes at a significant cost to the grower and we estimate that 15–20% of the crop is lost due to late blight.

Overall Impact of Phytophthora in Southeast Asia

In this chapter, we have tried to give a realistic picture of disease losses experienced due to Phytophthora in seven major crops that are grown on a large scale in Southeast Asia. Disease impact varies between varieties, cropping methods, regions, seasons, and years, and our overall disease assessment gives no more than a sweeping overview of the situation.

If we combine all disease assessments in Table 2.8 and add up the subsequent disease losses, we come to an average figure of USD2.4 billion for disease losses, with a minimum of USD2.1 billion and a maximum of USD2.7 billion. These figures are derived from the sum of losses for each country for the seven crops under discussion (Table 2.9). Disease losses for *P. infestans* in potatoes and *P. sojae* in soybean at a global scale have been estimated at USD3 billion and USD1.2 billion per annum, respectively.

Table 2.8 Summary of losses (USD '000) due to Phytophthora in seven main crops in five Southeast Asian countries.

Country	Minimum	Maximum	Average
Indonesia	639,272	886,444	762,859
Malaysia	295,949	399,111	347,531
Philippines	181,203	247,413	214,308
Thailand	617,412	828,041	722,727
Vietnam	351,249	386,433	368,841
Total	2,085,085	2,747,442	2,416,266

In addition to the crops outlined here, there are a large number of important tropical crops that also suffer from Phytophthora. We know that significant disease losses are experienced in tomato, tobacco, vanilla, eucalypt forestry, papaya, longan and chilli pepper.

Another important aspect of diseases is that they increase the risk of production of crops. Many smallholders have severe credit restrictions. In practical terms, this means that they do not have the funds to buy inputs to control and manage diseases. Thus, these smallholders are exposed to epidemics and risk large losses. Some *Phytophthora* diseases may also kill mature trees such as durian, citrus and cocoa, severely reducing the production capability

of the small holder. Hence, in order to reduce losses due to *Phytophthora* we need to provide cheap and effective disease control methods that can be adapted with very little inputs. In addition more resistant germplasm is needed and made available to small holders to reduce the enormous impact currently imposed on smallholders by *Phytophthora* pathogens.

Table 2.9 Details of losses due to *Phytophthora* in seven different crops in five different countries in Southeast Asia.

		USD '000				
		Value	Disease loss (%)	Minimum loss	Maximum loss	Average loss
Cocoa	Indonesia	788,952	15–20	118,343	157,790	138,067
	Malaysia	88,268	15–20	13,240	17,654	15,447
	Philippines	11,112	15–20	1,667	2,222	1,945
	Thailand	741	15–20	111	148	130
	Vietnam	na	15–20	-	-	-
	Total			133,361	177,815	155,588
Durian	Indonesia	780,000	20–25	156,000	195,000	175,500
	Malaysia	1,020,000	20–25	204,000	255,000	229,500
	Philippines	522,000	20–25	104,400	130,500	117,450
	Thailand	2,686,000	20–25	537,200	671,500	604,350
	Vietnam	330,000	20–25	66,000	82,500	74,250
	Total			1,067,600	1,334,500	1,201,050
Rubber	Indonesia	839,204	5–10	41,960	83,920	62,940
	Malaysia	521,201	5–10	26,060	52,120	39,090
	Philippines	11,756	5–10	588	1176	882
	Thailand	986,268	5–10	49,313	98,627	73,970
	Vietnam	250,000	5–10	250,000	250,000	250,000
	Total			367,921	485,843	426,882
Coconut	Indonesia	140,069	10–15	14,007	21,010	17,509
	Malaysia	2789	0–5	0	139	70
	Philippines	686,000	10–15	68,600	102,900	85,750
	Thailand	2870	0–5	-	144	72
	Vietnam	1100	0–5	-	55	28
	Total			82,607	124,248	103,429
Pepper	Indonesia	191,241	5–10	9562	19,124	14,343
	Malaysia	106,783	5–10	5339	10,678	8009
	Philippines	224	10–15	22	34	28
	Thailand	3082	10–15	308	462	385
	Vietnam	103,000	15–20	15,450	20,600	18,025
	Total			30,681	50,898	40,790

Table 2.9 (Cont'd) Details of losses due to Phytophthora in seven different crops in five different countries in Southeast Asia.

		USD '000				
		Value	Disease loss (%)	Minimum loss	Maximum loss	Average loss
Citrus	Indonesia	260,000	6–12	15,600	31,200	23,400
	Malaysia	11,000	6–12	660	1320	990
	Philippines	67,000	6–12	4020	8040	6030
	Thailand	413,000	6–12	24,780	49,560	37,170
	Vietnam	172,000	6–12	10,320	20,640	15,480
	Total				55,380	110,760
Potato	Indonesia	1,892,000	15–20	283,800	378,400	331,100
	Malaysia	311,000	15–20	46,650	62,200	54,425
	Philippines	12,704	15–20	1906	2541	2223
	Thailand	38,000	15–20	5700	7600	6650
	Vietnam	63,190	15–20	9479	12,638	11,058
	Total				347,535	463,379

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3

Biology of *Phytophthora*



3.1 *Phytophthora* in the Tropics

André Drenth¹ and David I. Guest²

Abstract

Most of the 60 described *Phytophthora* species are important in temperate as well as tropical regions. The various species, and in some cases the same species, can cause a wide array of different diseases on the same and on different crops. An understanding of typical symptoms is therefore important to recognise phytophthora disease problems in the field. An understanding of the evolutionary placement, life cycle and disease cycle of *Phytophthora* is paramount to developing sustainable disease-control strategies. Phytophthora diseases impose major limitations on the productivity and viability of many tropical and subtropical crops. Effective management of these diseases need to be based on a sound understanding of the biology of the pathogen, including its modes of survival and dissemination, host range and the role of environmental factors in the disease cycle. Examples in these proceedings, drawn from research on phytophthora diseases of cocoa, coconut, durian and other hosts, illustrate these points.

The Genus *Phytophthora*

Phytophthora de Bary 1887 is a cosmopolitan genus of Oomycete obligate plant pathogens containing approximately 60 described species (Erwin and Ribeiro 1996). The *Phytophthora* genus is a member of the Order Peronosporales within the Phylum Oomycota. *Phytophthora* species attack a wide range of plants, and are responsible for some of the world's most destructive plant diseases – examples include the European potato famine of the 19th century caused by *P. infestans* (Bourke 1964). Phytophthora diseases have been well studied in the temperate regions of the world. However, they are very common also throughout the wet tropical regions of the world and cause significant disease losses in many tropical fruit crops in the form of root rots, collar rots, stem cankers, leaf blights and fruit rot. *P. palmivora* alone, for example, causes a myriad of severe diseases on many different crops including: black pod of cocoa; root, stem and fruit rot of pawpaw; root rot and fruit rot of citrus; bud

rot in palms; black stripe in rubber; and root rot, trunk canker, and fruit rot in durian.

Evolutionary Placement

There has been considerable debate in the 20th century about the evolutionary placement of Oomycetes. First they were placed in the Fungal Kingdom but then moved to the Protists followed by the Kingdom Chromista, recently renamed to the Stramenopiles Kingdom (Hawksworth et al. 1995; van de Peer et al. 1996; Beakes 1998) (Table 3.1.1). The Oomycetes share many characteristics of ecology and life history with the true fungi. However, they are clearly distinguished from the Basidiomycetes and Ascomycetes by their genetics and reproductive mechanisms (Erwin and Ribeiro 1996). Their placement in the Kingdom Chromista (Cavalier-Smith 1986) and later the Stramenopiles was supported by a large number of characteristics, including variation in metabolic pathways (Hendrix 1970; Wang and Bartnicki-Garcia 1973; Elliott 1983), the presence of β -glucans rather than chitin in cell walls (Bartnicki-Garcia and Wang 1983), production of motile heterokont zoospores (Desjardins et al., 1969), and predominance of the diploid stage in the lifecycle (Erwin and Ribeiro 1996). The Oomycetes includes four orders, two of which, the Saprolegniales and the Peronosporales, contain important plant pathogens. The other two orders

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contain small groups of mainly aquatic fungal-like organisms. Within the Peronosporales, the family Pythiaceae contains a number of genera, the best known of which are *Phytophthora* and its sister group, *Pythium*, a genus of approximately 120 species (van der Plaats-Niterink 1981).

Phytophthora as Plant Pathogens

Almost all species within the genus *Phytophthora* are formidable plant pathogens. Hence, we have to ask the question: What makes these organisms such effective plant pathogens? The following factors are involved:

- The ability to produce different types of spores such as sporangia and zoospores for short-term survival and spread, and chlamydospores and oospores for longer term survival.
- Rapid sporulation on host tissue within 3–5 days of infection. This results in a rapid build-up of secondary inoculum in a multicyclic fashion, leading to epidemics under suitable favourable environmental conditions.
- Ability of zoospores of *Phytophthora* to be attracted to root tips through a chemical stimulus (positive chemotaxis) as well as root-generated electric fields (electrotaxis) (van West et al. 2002), coupled with the mobility of zoospores to actually swim to the actively growing root tips, encyst, and infect young, susceptible root tissue.
- Ability to survive in or outside the host tissue as oospores or chlamydospores for long periods. Oospores are also known to survive passage through the digestive systems of animals such as snails.
- Production of sporangia, which can be airborne and may travel reasonable distances in raindrops, run-off and irrigation water, and on wind currents, to infect neighbouring fields. These sporangia can directly infect host tissue. These same sporangiospores also have the ability to differentiate into 4–32 zoospores under humid

and cool conditions and cause multiple infections from the one sporangium. Nevertheless, zoospores can travel only short distances, as they are susceptible to desiccation.

- *Phytophthora* pathogens belong to the Kingdom Stramenopiles and as such have different biochemical pathways to the true fungi. Many fungicides are therefore not very effective against phytophthora pathogens.
- *Phytophthora* pathogens thrive under humid and wet conditions, which makes them difficult to control, as protectant fungicides are difficult to apply and least effective under such conditions.

Symptoms of Phytophthora Diseases

Phytophthora pathogens can cause many different diseases and disease symptoms on a wide range of plant species. In the next section, the disease symptoms most often encountered are discussed.

Root rot

Seedlings of many plants are very susceptible to root rot and damping off caused by phytophthora. The early symptoms are the wilting and yellowing of young seedlings. General symptoms of root rot are that plants appear water stressed, chlorotic, and are often stunted in their growth. New leaves are often small and show a light green to yellow colour and wilting occurs even in the presence of sufficient water. Affected root tissue is soft, watersoaked and discoloured to dark brown rather than the creamy white colour of healthy roots. Advanced root rot leads to the lack of secondary and tertiary roots and a lack of healthy root tips (Figure 3.1.1).

Collar rot

Collar rot, sometimes called foot rot, often develops at or just below ground level. The infection frequently moves upwards from the roots, rotting the lower bark tissue and discolouring the lower stem. Exudation of gum often occurs in the affected parts. The affected bark area is often irregular in shape and size and first

Table 3.1.1 Classification of the Oomycetes (Hawksworth et al. 1995; Beakes 1998).

Kingdom	Class	Order	Family	Genus
Stramenopiles	Oomycetes	Lagenidiales	Saprolegniaceae	<i>Achlya</i>
		Leptomitales		<i>Saprolegnia</i>
		Saprolegniales		<i>Pythium</i>
		Peronosporales		<i>Phytophthora</i>
			Peronosporaceae	<i>Bremia</i>
			Albuginaceae	<i>Peronospora</i>
				<i>Albugo</i>

appears as a water-soaked lesion, before drying, becoming sunken and giving rise to cracks in the bark that usually show dark-brown discoloration. Above-ground symptoms appear as wilting, reduction of foliage, and dieback of branches such as the symptoms caused by *P. capsici* in pepper (Figure 3.1.2). Bark and cortex tissues often have a swollen and cracked appearance, separating easily from the underlying tissue. The disease may also progress around the trunk giving rise to girdling of the main roots or the trunk.



Figure 3.1.1 Roots of pineapple affected by *Phytophthora cinnamomi*.

Tree canker

Many species of *Phytophthora* can form cankers on the stems of host plants. These cankers have various names, including stripe canker (cinnamon), patch canker (durian) or trunk canker (cocoa). The first sign of canker is usually the appearance of wet lesions on the bark surface (Figure 3.1.3), often close to the branch points at the lower end of the trunk. Bark discoloration and exudation of reddish



Figure 3.1.2 (Left) Wilting of pepper due to *Phytophthora capsici*. (Right) Section of the main root affected by *P. capsici*.

brown, resinous substance frequently accompany necrosis. When the bark is stripped away, the cortical tissues and wood appear dull and discoloured from cream coloured to reddish brown (Figure 3.1.4). Wood lesions are often very irregular in shape but are well defined. Expanding lesions severely restrict water and nutrient flow to the connecting branches, leading to wilting. If the lesion girdles the tree branch, dieback is more widespread in the crown and the tree may lose all its leaves.



Figure 3.1.3 Lesion on the bark of cocoa tree due to *P. palmivora*.

Stem lesions

Some species of *Phytophthora* attack leaves as well as stems. For example, *P. infestans* on potato and tomato, and *P. nicotianae* on tobacco. In advanced stages, dry, dark-brown or black lesions develop in the cortical tissue on the stem. Lesions frequently start near the soil line and subsequently expand upward and may cover as much as half the length of the stem in the case of black shank on tobacco. Expanded lesions often girdle the stem and give rise to wilting and death of the upper branches and leaves.





Figure 3.1.4 Reddish brown canker on cocoa tree caused by *Phytophthora palmivora*.



Figure 3.1.6 Bud rot symptoms in coconut palm caused by *Phytophthora palmivora*.



Figure 3.1.5 Stem lesion in tomato caused by *Phytophthora infestans*.



Figure 3.1.7 Lesions of *Phytophthora palmivora* on the heart of a bud rot affected palm.

Young immature stems are often most susceptible, as in stem blight of tomato caused by *P. infestans* (Figure 3.1.5).

Bud rot

Bud rot (sometimes called heart rot), is a serious problem in many species of palms. It is caused predominantly by *P. palmivora*. The symptoms of bud rot of palm are exhibited over a period of months, often following severe storms, which facilitate infection and spread of phytophthora. Symptoms first appear as discolouration and wilting of the spear leaf and one or more of the newest

leaves, which become chlorotic (Figure 3.1.6). These new leaves may exhibit lesions from infection that has occurred in the spear. As the infection in the bud of the palm progresses, newly emerging leaves show increasing amounts of damage. Eventually, the spear leaves can be pulled out easily because they are rotted at the base, where some white mycelial growth may be observed. The fronds will turn yellow, then brown, and will fall off, finally leaving only a naked, dead trunk. In the base of the bud, small lesions can be seen (Figure 3.1.7), but secondary invaders soon move in, and fluid starts to collect giving off a foul smell. The tissue below the

bud shows discolouration from reddish brown to brown. It is hard to isolate *Phytophthora* from palms with advanced bud rot due to bacterial decay of the bud. Trees that are beginning to show symptoms with an advancing margin on the bud should be used instead, as they are often still relatively free of secondary invaders.

Heart rot

A number of *Phytophthora* species cause heart rot, but a common one in tropical regions is heart rot of pineapple caused by *P. nicotianae* and *P. cinnamomi*. Young pineapples with heart rot show chlorotic foliage and necrotic leaf tips (Figure 3.1.8). The heart leaves towards the centre of the plant are easily pulled out and show rotting at the base with a characteristic delimited brown lesion indicating the growth of the pathogen (Figure 3.1.9). Under wet conditions, a foul odour accompanies the rotting of the base of the leaves and invasions of secondary pathogens. Heart rot is most common on young plants, while older plants may show restricted lesions slightly higher up the stem.



Figure 3.1.8 Symptoms of heart rot in pineapple caused by *Phytophthora nicotianae*.

Leaf blight

Several *Phytophthora* species cause leaf blight. These include *P. infestans* on potato and tomato, *P. palmivora* on rubber and a large number of tropical fruit species including durian (Figure 3.1.10), and *P. colocasiae* on taro (Figure 3.1.11). These blights on leaves are first seen as small flecks, but within 3–5 days they expand to produce large lesions. Initially, infected tissue is watersoaked but becomes necrotic (brown or black) in a few days. Often the lesions are surrounded by a halo of light green tissue. Spores appear as white velvety growth at the edge of the lesions, primarily at the underside of the leaf. It is this white growth that distinguishes phytophthora

leaf blight from several other foliar diseases. Large amounts of sporangiospores are often produced as 1–4 sporangiophores extend from the stomata at the underside of the leaf. Sporangiospores can become airborne and lead to rapid spread of the disease.



Figure 3.1.9 Brown lesions on the bottom of pineapple leaves affected by heart rot.

Fruit rot

Fruit rot caused by *Phytophthora* species is common in a large number of different plant species, including citrus, durian, cocoa, papaya and chilli pepper. It appears as watersoaked lesions with light-brown centres 3–5 days after infection, depending on the host. The lesions expand rapidly and can completely rot an entire fruit. Under conditions of

high humidity, white/grey mycelium may be found behind the advancing margin of the lesions (Figure 3.1.12). Often the fruit does not drop and may mummify on the tree. The infection can also be internal, as in the case of *P. palmivora* in papaya where mycelial growth can be seen on the seeds after cutting open infected fruit. Brown rot on citrus develops as an expanding circular lesion with a dull-brown colour. Typical of many fruit rots caused by phytophthora is that the diseased tissue remains firm as it darkens in colour. In the case of brown rot in citrus, a strong odour coming from the fruit is another characteristic of the disease.

Tuber and corm rot

Tubers of potato and corms of taro are considered to be enlarged stem pieces and are susceptible to infection by phytophthora. Potato tubers can be infected by zoospores of *P. infestans* washed down by rain from the leaves. Tuber infections are characterised by patches of brown to purple discolouration on the potato skin (Figure 3.1.13). Cutting just below the skin reveals a dark, reddish-brown, dry corky rot. Heavy infection



Figure 3.1.10 Lesion of *P. palmivora* on durian leaf.



Figure 3.1.11 *P. colocasiae* lesion on taro leaf.

can give rise to severe rot and total loss of the tubers. Light infections can occur and are difficult to detect. However, if such potatoes are used as seed potatoes they can infect the emerging stems and start off a new epidemic in the next planting season. This is probably how most late blight epidemics start. Potato can also be infected by *P. erythroseptica*, causing the so-called pink rot disease. Infected tubers have a dull brown appearance and exude water under pressure. The cut surface of tubers becomes faint pink after exposure to air. After 30 minutes, the entire cut surface of the tuber turns bright pink. If corms of taro are infected with *P. colocasiae*, they stay firm and leathery, which is typical of phytophthora dry rot. Under favourable conditions, the corms may rot completely after about one week.

Life Cycle

The life cycle of *Phytophthora* may involve up to three asexual spore forms – sporangia, zoospores, and chlamydospores – in addition to oospores, the sexual spore form (Figure 3.1.14). Diploid vegetative mycelium produces asexual sporangia, which may germinate directly, or differentiate to produce 8–32 zoospores, each of which passes through a cycle of dispersal and encystment before germinating. Some species, such as *P. cinnamomi*, also produce significant numbers of asexual chlamydospores from the mycelium. Sexual reproduction results in the production of oospores. All spore types are potentially infective, and chlamydospores and oospores also function as overwintering or resting structures. All species of *Phytophthora* have a soil-borne resting stage. In addition, some species, such as *P. palmivora*, are also aerially dispersed, primarily as caducous (deciduous) sporangia.

Host Range

Species of *Phytophthora* vary greatly in their degree of host specificity. *P. fragariae* var. *rubi* infects a single host species (Kennedy and Duncan 1995), while *P. cinnamomi* is able to attack over 1000 different host-plant species (Erwin and Ribeiro 1996), and other species lie in the range between these two extremes.

In the tropics, the most commonly encountered *Phytophthora* species is *P. palmivora* which has a large host range. *P. nicotianae* is also common and occurs on many different host species. *P. capsici* has a slightly more restricted host range but is still able to infect over 40 different crop plant species. *P. hevea* and *P. katsurae* are considered to have a narrow host range when it comes to crop plants, but are commonly found in some samples obtained from

rainforest soils. Some *Phytophthora* species in the tropics are very host specific, such as *P. colocalisae* on taro. It is not difficult to understand that control of, for example, *P. palmivora* on a particular crop involves a far more complex approach that has to involve the alternative hosts, than, say, control of *P. colocalisae* on taro which has a very restricted host range (Erwin and Ribeiro 1996; Zentmyer 1980).

Mating System

All isolates of *Phytophthora* are potentially bisexual; that is, they are able to produce both male and female sexual structures, or gametangia (Galindo



Figure 3.1.12 Advancing lesion of *P. palmivora* on durian fruit. Note the white sporulation in the centre of the lesion

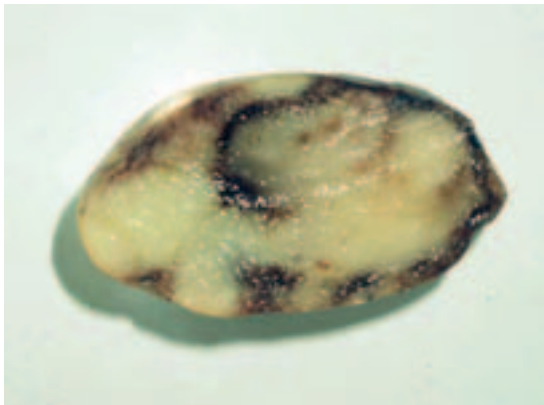


Figure 3.1.13 Tuber infection of potato caused by *P. infestans*.

and Gallegly 1960). However, only about half of the species of *Phytophthora* are homothallic, and able to produce oospores rapidly and abundantly in single culture. The remaining species are heterothallic, and produce gametangia only in response to chemical stimulation from an isolate of the opposite mating type (Brasier 1992; Ko 1978).

The system of heterothallism involving A1 and A2 mating types is universal throughout the genus. Isolates of opposite mating types from different species are often able to reciprocally stimulate gametangial formation (Ko 1978). The mating system of a *Phytophthora* species determines its ability to outbreed: homothallism allows frequent selfing, whereas heterothallism encourages outbreeding. However, both homothallic and heterothallic species do have a range of reproductive options. Homothallic species have recently been shown to undergo low levels of outbreeding in vitro (Whisson et al. 1994), while heterothallic species have been shown to inbreed at low levels (Goodwin et al. 1994).

Sexual reproduction has a number of roles in the life cycle of phytophthora. It allows for recombination of the existing alleles in the case of heterothallic *Phytophthora* species, while for both homothallic and heterothallic species, oospores may act as a structure permitting survival for long periods in the absence of a host plant. Oospores may also remain in infected host tissue to overcome adverse conditions for further colonisation such as hot and dry weather.

At present we do not know the relative importance of sexual reproduction in most *Phytophthora* species. Although the role of oospores in the epidemiology has to a large degree been evaluated for *P. infestans* in temperate regions, the role of sexual reproduction and the formation of oospores in the tropics are not well understood for any *Phytophthora* species.

Morphological Variation

Details of the morphological properties and pathology of many of the 60 described species of *Phytophthora* are collated in Erwin and Ribeiro (1996). In traditional taxonomy, species were discriminated mainly on the structure of the sporangium (non-papillate, semi-papillate, or papillate), the form of the antheridium (amphigynous or paragynous) and on whether the taxon is inbreeding (homothallic), or outbreeding with A1 and A2 sexual incompatibility, or mating types (heterothallic) (Tucker 1931; Waterhouse 1963). Heterothallic taxa are exclusively amphigynous while homothallic taxa may be

amphigynous, paragynous or, in some cases, have antheridia of both types. Waterhouse (1963) assigned *Phytophthora* taxa to six morphological groups which have provided the framework for a number of traditional identification keys (e.g. Stamps et al. 1990).

Many researchers have observed that there are considerable levels of morphological variation within and between *Phytophthora* species, which makes identification of some isolates to species level difficult. A number of *Phytophthora* species have been reclassified over the years. Numerous changes have taken place especially in the *P. megasperma* species complex. A number of species were morphological indistinguishable from the complex although this was recognised and the use of *formae speciales* promoted (Hansen and Maxwell 1991).

P. megasperma was first described by Drechsler (1931). This first description was later broadened (Tompkins et al. 1936). In the 1950s a disease found on soybean in Illinois was designated as being caused by a new species. Since the morphology of this species was highly similar to the previously described *P. megasperma*, Hildebrand (1959) renamed it *P. megasperma* var. *sojae* as it showed high levels of host specificity towards soybean. In a reevaluation of the species, Kuan and Erwin (1980) showed a continuous distribution of oogonial size between the various

varieties within the *P. megasperma* complex and proposed the use of *formae speciales*. Since no simple morphological character distinguished the different *formae speciales*, this system was used quite extensively. However, with the advent of molecular taxonomy, the genetic relationships between the various species within this complex were tested and it was shown that various distinct biological species were lumped together in the *P. megasperma* species complex (Forster et al. 1989). The taxonomic status was subsequently reviewed by (Hansen and Maxwell 1991); one species was (*P. sojae*) reinstated and two others (*P. medicaginis* and *P. trifolii*) were created. The genetic relationship between these species is confirmed and illustrated in a more recent phylogeny of the genus *Phytophthora* (Cooke et al. 2000).

Phytophthora palmivora, which is probably the most important *Phytophthora* species in the tropics, also has undergone several changes in classification since Butler (1919) first described it. *Phytophthora palmivora* shows considerable morphological and pathological variation and, since the original description, a number of additions and delineations have been proposed. First *P. palmivora* strains were grouped together based on the host from which they were collected (Gadd 1924). This sometimes correlated with mating type, giving rise to further confusion, as reviewed by Zentmyer et al. (1977).

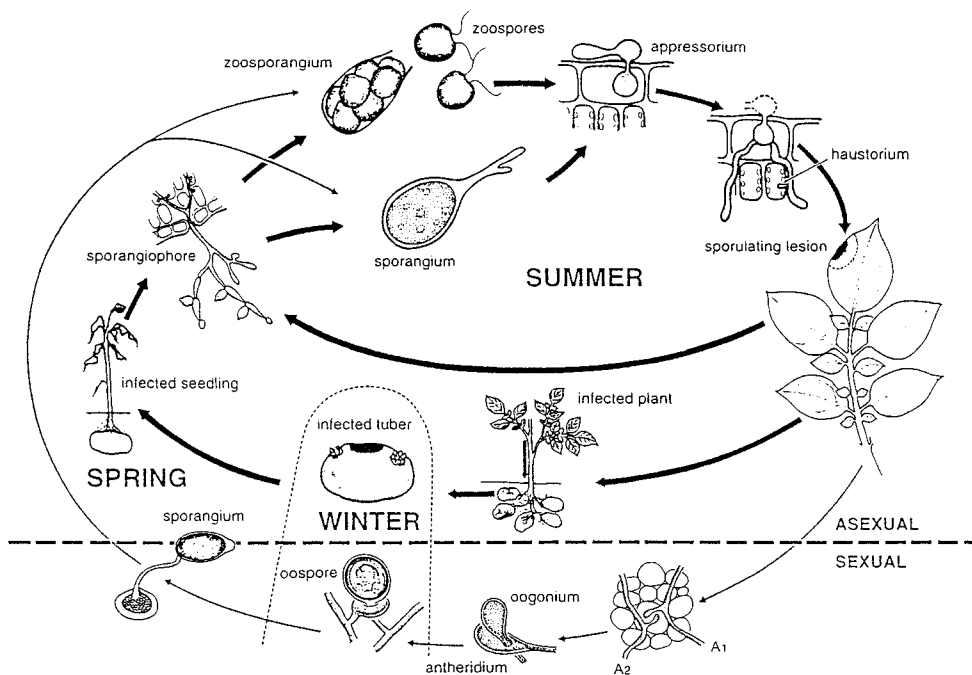


Figure 3.1.14 Life cycle of *Phytophthora infestans*. Reproduced from Dreth (1994).

Once it became clear that species other than *P. palmivora* could infect cocoa, and that each of these had a different and sometimes extensive host range (Brasier et al. 1981), a better delineation of species began to emerge. The species was first split into four different morphological groups, MF1–MF4. MF1 was the typical form of *P. palmivora*, while MF2 was, for some time, deemed to be morphologically different. It was later found to be insufficiently different and lumped with MF1 again. MF3 was renamed *P. megakarya* (Brasier and Griffin 1979) based on pedicel length and chromosomal differences. MF4 was found to be closely related to *P. capsici* and thus this group was placed in the redescribed species *P. capsici* (Tsao 1991). These species reclassifications were later confirmed in the evolutionary analysis of the *Phytophthora* genus by (Cooke et al. 2000).

The above two examples illustrate the significant amount of variability in morphological and physiological characters that have to be taken into account when trying to classify organisms. From the outset one does not know the extent of variation present, while the boundaries that define biological species are not always straightforward. If we study only a few isolates of a few species at any point in time we are trying to complete an evolutionary jigsaw puzzle while holding only a few of the pieces. Determining where these few pieces go in this evolutionary puzzle often turns out a difficult task. It is clear that without large collections of the material from different hosts and regions under investigation, and an understanding of variability within and between species, it is difficult to resolve these matters. In the past decade, molecular taxonomy has provided an enormous insight into phylogenetic relationships between the various species. This has allowed testing of hypotheses concerning the delineation of difficult species complexes.

Disease Cycle

Primary inoculum

Phytophthora is basically a soil-borne organism, although species including *P. palmivora* are well adapted to attack aerial parts of plants causing diseases such as cankers, leaf blights and fruit rots. Primary inoculum initiates epidemics when environmental conditions are conducive. In the monsoonal tropics, this usually means the wet season, but in the wet tropics conditions conducive to the development of phytophthora diseases may persist throughout the year, enabling an unbroken disease cycle.

Primary inoculum of *Phytophthora* spp. survives as mycelium and chlamydospores in infected roots, soil, bark cankers and mummified fruits or pods. For example, unharvested, infected cocoa pods become mummified, develop sporangia during the rainy season and drop inoculum onto pods below every time it rains, for up to three years. Untreated bark and flower cushion cankers also develop and release sporangia that are carried in run-off water down the stem. Although both mating types are often present, oospores are relatively rarely formed in tropical species of *Phytophthora*. The role of oospores as a source of inoculum of heterothallic species, such as *P. palmivora*, in the tropics is poorly understood.

Secondary inoculum

Once conditions conducive to the disease are present, primary inoculum germinates and establishes an infection. If this infection succeeds, a generation of secondary inoculum is produced which fuels propagation of the epidemic. The rate of propagation and the success of these propagules in causing new infections determines the slope of the disease progress curve — explosive epidemics are caused by the rapid increase in secondary inoculum. For example, although it only takes a single zoospore to initiate the infection of a cocoa pod, the lesion spreads rapidly and will release 4 million sporangia from a single pod within a week (Medeiros 1976). Sporangia also form on infected debris and roots on the surface of soil, and are released into water pooling on the surface, or into creeks, rivers and dams.

Sporangia are dislodged by water, wind, rapid changes in humidity or by contact with vertebrate or invertebrate vectors. Sporangia of many species germinate in the presence of free water, either in the soil, in ponds, or on films of water on aerial plant surfaces, to release around 30 zoospores. Zoospores swim and are attracted chemotactically and electrotactically to suitable penetration sites, such as stomata or anticlinal wall junctions. Zoospores may remain motile for several hours, but usually encyst within 30 minutes if host tissues are present. Encystment involves shedding of the flagella and the rapid deposition of a cell wall around the zoospore. Cysts germinate to form a germ tube that is also tactically attracted to suitable penetration sites.

While indirect germination of sporangia, through the release of zoospores, is common, some species may also germinate directly to form a germ tube. If no suitable hosts are located, a secondary sporangium may form. If host tissue is located, the germ tube forms an appressorium that attaches to

the host surface, then penetrates and infects. Successful colonisation results in the development of further infective or resting propagules.

Movement of inoculum

A close examination of disease symptoms can give valuable insights into the biology and disease cycle of the pathogen. For example, phytophthora lesions may be initiated on various parts of a cocoa pod (Figure 3.1.15). Lesions beginning at the peduncle reflect either direct contact with a stem canker lesion, or with ant tents constructed with contaminated soil. Lesions beginning at the distal end of the pod indicate that the inoculum was borne in drops of water contaminated with pathogen propagules, most likely originating from pod mummies or stem cankers higher in the canopy, or from soil-splash on pods close to the ground. Lesions beginning on the side of pods are mostly associated with damage caused by flying insects, mammals or knife wounds.



Figure 3.1.15 Naturally occurring *P. palmivora* pod rot on cocoa showing lesions initiated at (from left to right) the distal end, the peduncle end and at the pod equator.

This simple analysis reveals several sources of inoculum and several modes of dissemination of *P. palmivora* within cocoa canopies in Papua New Guinea. Inoculum moves from the soil into the canopy as a result of human activity, rain and soil-splash, tent-building ants, termites, slugs and flying beetles. The beetles breed in discarded pod cases, visit flowers and are attracted to pod lesions (Konam and Guest 2004). When they bore into pod lesions they release large amounts of easily dispersed, contaminated frass. Once in the canopy, secondary inoculum spreads to infect pods, cankers, flower cushions, leaves and chupons. Secondary inoculum moves to pods by direct contact, contaminated

implements, raindrops, ants, flying beetles and mammals.

Black stripe and patch canker of rubber caused by *P. palmivora* presents an unusual situation. The tapping operation creates a wound that facilitates pathogen entry, especially if the panel is close to soil splash from the ground. The tapping knife itself provides another means for spreading secondary inoculum from tree to tree.

Footrot, cankers, gummosis, seedling and leaf blights are commonly initiated by soil splash inoculum, where raindrops dislodge sporangia and zoospores on the soil surface or in pools and puddles of water onto the base of the stem and low-lying leaves. Root rots and root cankers are almost always initiated by the migration of zoospores in the soil water.

Environment

The activation of primary inoculum, production and release of secondary inoculum and infection all depend on humidity and free moisture. Although symptoms appear year-round, the most severe epidemics coincide with the proliferation of sporangia and insect vectors during the wet season. Zoospores generally need 20–30 minutes in free water on the plant surfaces for the start of encystment and germination; then, given sufficient atmospheric moisture, those that have germinated will continue to grow. If susceptible plant surfaces remain wet for several hours, there is a high probability of infection if zoospores are present. Temperature rarely limits the development of phytophthora diseases in the tropics, other than in highland environments.

Implications for disease management

Phytophthora disease cycles are complex, involve numerous sources of primary and secondary inoculum and several modes of dissemination. As an organism it is flexible and very well adapted to monsoonal and wet tropical environments.

Integrated disease management strategies should address numerous components of the disease cycle by selecting disease-resistant planting material, and preventing or disrupting the dissemination of primary inoculum from the soil into the canopy and the movement of secondary inoculum from one part of the canopy to another. Mixed plantings of genetically diverse plants, that include medicinal plants, herbs, fruit, vegetables and timber trees, may prevent rapid inoculum build-up and sustain farm productivity over a longer period. Treatments that increase soil microbial activity reduce the survival of

chlamydospores and mycelium in infected debris. Postharvest disease can be suppressed through fungicide treatments and low temperature storage.

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3.2 Infection Biology of *Phytophthora palmivora* Butl. in *Durio zibethinus* L. (Durian) and Responses Induced by Phosphonate

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Abstract

We investigated the infection biology of *Phytophthora palmivora* on durian leaf and fruit. Zoospores of *P. palmivora* are preferentially attracted to fresh wounds in durian and such wounds are shown to be key infection courts. Overlapping layers of peltate trichomes cover the stipules, lower surface of the leaf, petiole, young stem and fruit, and are the first point of contact between the pathogen and the host on these tissues. The pathogen binds randomly to the surface of the trichomes but is unable to penetrate the heavily lignified walls, however the hypha can grow over the edge of the trichome until it reaches the epidermal surface beneath. The stomata that occur beneath the trichomes on all tissues are readily infected by the advancing hyphal strands, and are also major infection courts. When infection occurs through fresh wounds in leaves, lesions appear within 2 days and leaves are entirely diseased within 6 days. Treatment of durian seedlings with phosphonate before inoculation with *P. palmivora* led to a significant restriction of the pathogen, but only if leaves were inoculated while still attached to the tree, and not if they were excised before inoculation. Phenylalanine ammonia lyase activity was not stimulated in excised inoculated leaves from phosphonate-treated durian seedlings, compared to untreated seedlings.

Introduction

An understanding of the infection biology of a host/pathogen interaction is essential in understanding the disease cycle, and ultimately in formulating effective disease management strategies.

Phytophthora palmivora Butl. is the most important pathogen of durian (*Durio zibethinus* L.), but there is no readily available information on the processes of

infection. However, there is a wealth of published information on a number of other phytophthora ‘pathosystems’ that cause significant economic and ecological damage. It has been repeatedly demonstrated in these other systems that morphological, anatomical and biochemical characteristics of the host largely determine the outcome of an encounter with the pathogen. The physical characteristics of the host become even more important when there is susceptibility to *Phytophthora* spp. with caducous sporangia, as is the case with durian and *P. palmivora*, as the pathogen has access not only to the root zone but to all the aerial tissues.

Chemical control of plant diseases is moving away from a total dependence on fungicides to the use of systemic compounds that alter the biochemistry of the interaction between host and pathogen, inducing the plant’s natural defence responses. One

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such compound, 'phosphonate' (salts or esters of phosphonic acid), has proven highly successful in controlling phytophthora diseases in a number of crops including avocado (Darvas et al. 1984), cocoa (Holderness 1990; Guest et al. 1994) and, more recently, black pepper (see Chapter 7.4) and durian (see Chapter 8.5). The mode of action of phosphonate is thought to be due to the disruption of phosphate metabolism in the pathogen, which causes the release of pathogen stress metabolites that activate host defence responses (Guest and Grant 1991). A key step in plant defence responses is activation of the enzyme phenylalanine ammonia-lyase (PAL) that is involved in the biosynthesis of phenylpropanoids, including phytoalexins, salicylic acid, lignin and suberin.

The major objective of the current study was to examine how the morphological and anatomical characteristics of durian influence infection by *P. palmivora*, and to investigate the effect of phosphonate on the infection process.

Methods

The results presented in this chapter were derived from a number of separate studies conducted at The University of Melbourne in Australia and Kasetsart University in Thailand. As Melbourne is well outside the climatic range for durian, seedlings were grown in a temperature-controlled glasshouse and the histopathological studies concentrated on shoot tissues. The biology of fruit infection was studied in Thailand where durian fruit was readily available. Axenic cultures of *P. palmivora* were used as inoculum in all studies, as it is the species predominantly associated with durian diseases in Australia and Southeast Asia (see Chapter 6.7).

Durian fruit was inoculated with a sporangial suspension (400 sporangia per cm² leaf) and incubated at 25°C and approximately 98% relative humidity. Shoot tissues were inoculated with a motile zoospore suspension, covered with a plastic bag to maintain a high humidity and incubated in a temperature-controlled glasshouse. For some studies, leaves were wounded by deliberate removal of trichomes with adhesive tape. Standard histological techniques were used for the preparation of samples for examination by either light or scanning electron microscopy.

Durian seedlings were treated with phosphonate by pouring 500 mL of a 1 g/L a.i. solution of Foli-R-Fos 200 (UIM Agrochemicals (Aust.) Pty Ltd) onto the surface of the potting mix. Trays were placed beneath the pots to capture any drainage, plastic bags were placed around the tray and pot and tied

around the main stem for 24 hours to minimise the loss of the liquid through drainage and/or evaporation. To determine the effect of phosphonate on symptom development, leaves were wounded (see Chapter 8.3) and the wound inoculated with sporangia, either while still attached to the tree, or after the leaves were excised. Excision of leaves (or other organs including fruit or stems) before inoculation is a standard bioassay technique for ranking resistance to infection in germplasm collections (see Chapter 8.3). Attached leaves were covered with a plastic bag and aerated each day when symptoms were monitored, while the seedlings were maintained in the glasshouse. Excised leaves were incubated in a humid chamber in a constant temperature cabinet at 28°C and symptoms monitored daily.

Leaves from phosphonate-treated durian seedlings were excised and inoculated with *P. palmivora* sporangia. Leaves were not wounded before inoculation. Activity of PAL was determined by measuring the amount of L-phenylalanine converted to cinnamic acid in extract from tissue immediately surrounding the region of inoculation, according to the methods of El Modafar et al. (2001).

As lesion development and changes in cinnamic acid concentrations over time were linear, the data were analysed by calculating the slope of the lines for each treatment by regression and comparing slopes by analysis of variance (ANOVA) (Minitab Inc., Version 14).

Surface Features of Durian and Their Influence on Pre-penetration Events

Motile zoospores of *P. palmivora* bind randomly and individually in low numbers to the smooth upper surface of the durian leaf, which has a continuous cuticle with no stomata or trichomes. The encysted zoospores readily germinate on the upper leaf surface but growth of the germ-tubes appears random, unlike growth at sites of preferential attraction as described below.

In *D. zibethinus*, trichomes occur on the lower leaf surface, petiole, young stem, the external surface of the stipule, and on fruit (except in the trough between the spines). Three distinct trichome types were identified on durian leaves: (i) glandular trichomes which are not lignified; (ii) stellate trichomes which vary in the level of lignification; and (iii) peltate trichomes which are heavily lignified and form the external layer (Figure 3.2.1) giving the lower leaf surface a silver to golden hue. Stomata occur in a random arrangement beneath the

trichomes on the petiole, young stem, lower leaf (although absent from the major veins) and fruit (although absent from the trough between the spines). There are no trichomes on durian roots.

A higher proportion of *P. palmivora* spores bind to the lower surface of the leaf, which is a function of the rough topography caused by the indumentum and trapping of spores at the ragged edges of the overlapping peltate trichomes. Under optimal environmental conditions, *P. palmivora* can bind, germinate, produce extensive hyphae and re-sporulate, thus completing its life cycle on the surface of the durian tissue within eight hours of inoculation (Figure 3.2.2).

Although successful penetration of heavily lignified peltate trichomes by *P. palmivora* was never observed, attempted penetration was marked by appressoria-like swellings and some dissolution of the trichome surface in the region of attachment (Figure 3.2.3). An unsuccessful attempt to penetrate was often followed by the formation of a hyphal branch from the swelling, growth of this hypha and attempted penetration at another site. This process could be repeated numerous times by a single zoospore/cyst (Figure 3.2.4). Invariably, some hyphae grow over the edge of the trichome and down to the surface of the tissue (Figure 3.2.5), where infection occurred through open stomata.

When trichomes were deliberately removed from the lower leaf surface *P. palmivora* did not show preferential attraction to the exposed stomata, and occasionally hypha grew across the stomatal pore with no attempt at penetration (Figure 3.2.6).

Phytophthora palmivora also showed no attraction to the axillary shoots of durian, probably due to the impressive trichome armour (Figure 3.2.7), which is already well developed on the leaf buds and external sides of the stipules before emergence.

Infection Courts of Durian and Penetration by *Phytophthora palmivora*

Although there is no evidence that *P. palmivora* zoospores are preferentially attracted to stomata, they are clearly important infection courts as, more often than not, hyphae will infect through open stomata as they grow with apparent randomness across the surface of the tissue (Figure 3.2.8).

Phytophthora palmivora is preferentially attracted to fresh wounds in durian tissue. When trichomes were deliberately removed from leaves, taxis of zoospores

to the resulting fresh wound was evident through heavy and localised spore binding, and docking of the cysts with the side of germ-tube emergence directed toward the wound (Figure 3.2.9). The demonstrated importance of fresh wounds as infection courts led to the investigation of wound healing in durian leaves. Using histological stains, suberin was detected in the remnants of the trichome stalk within 24 hours of trichome removal (Figure 3.2.10), while lignin and callose were detected within 48 hours. The intensity of lignin (Figure 3.2.11) and callose (Figure 3.2.12) staining increased with time, which coincided with a decrease in number of spores binding to the wound.

Phytophthora palmivora can directly penetrate the cuticle and epidermis on the upper surface of the durian leaf and in the trichome-free region between the spines of the durian fruit (Figure 3.2.13), usually at the anticlinal wall between epidermal cells.

Colonisation of Durian Tissues by *Phytophthora palmivora* and Symptom Development

Phytophthora palmivora rapidly colonised the entire leaf lamina when infection occurred through fresh wounds, and lesions were visible within 2 days of inoculation. The appearance of lesions resulting from a single *P. palmivora* isolate can be highly variable within and between trees, ranging from dark brown/black with a distinct margin to water-soaked light grey with a diffuse border.

When the pathogen infects through stomata of a durian leaf, colonisation is initially intercellular, particularly in the relatively open structure of, and surrounding, the sub-stomatal cavity. However, as the pathogen progresses through the leaf lamina into the more compacted mesophyll tissues, colonisation becomes increasingly intracellular (Figure 3.2.14).

Infection and symptom development in excised durian fruit did not occur unless high relative humidity (98%) was maintained for at least 72 hours after inoculation with *P. palmivora*.

When penetration and infection is successful, the pathogen proliferates within the host and sporangiophores exit either through stomata or by erupting through the epidermis (Figure 3.2.15), releasing a new generation of sporangia into the environment (Figure 3.2.16). In disease-affected durian orchards, this is often seen as a whitish bloom on severely infected organs, particularly fruit (Figure 3.2.17).

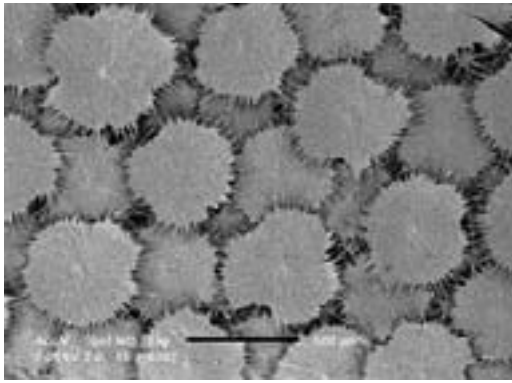


Figure 3.2.1 A scanning electron micrograph of the overlapping peltate trichomes that form the external layer of the underside of the durian leaf. Scale bar = 500 μm.

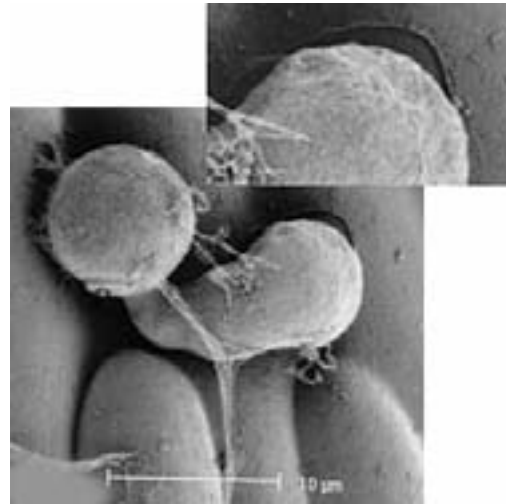


Figure 3.2.3 A scanning electron micrograph of a zoospore cyst of *Phytophthora palmivora* attempting to penetrate the surface of a lignified peltate trichome. The inset enlargement shows partial dissolution of the trichome surface (arrow). Scale bar = 10 μm.

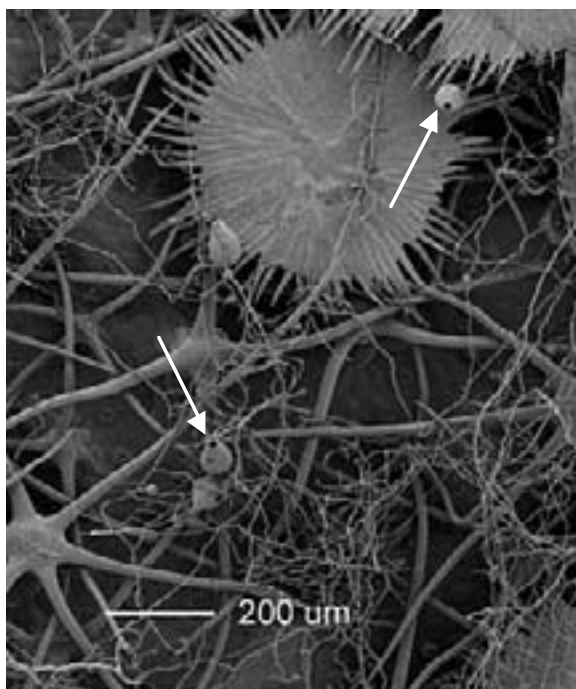


Figure 3.2.2 Proliferation of *Phytophthora palmivora* among the trichomes on the lower surface of the durian leaf. Sporangia have formed and the open exit pores (arrows) indicate that the zoospores have been released. Scale bar = 200 μm.

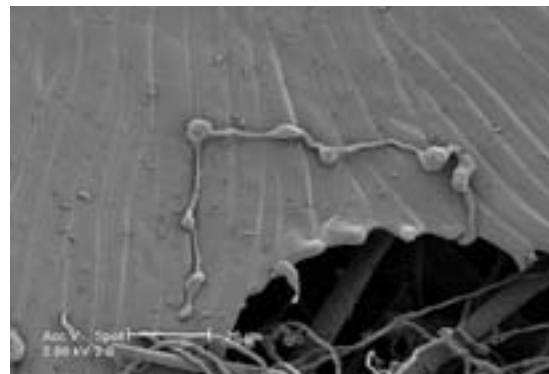
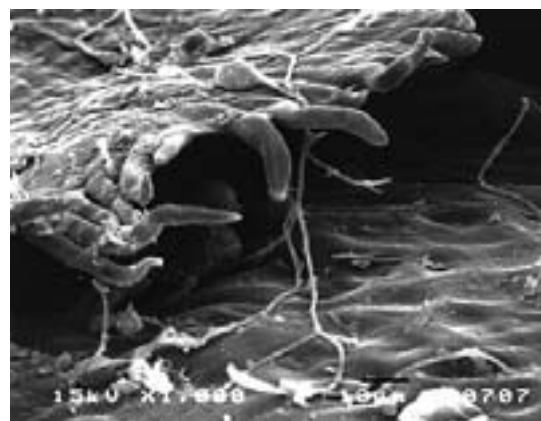


Figure 3.2.4 A hypha of *Phytophthora palmivora* makes repeated attempts to penetrate a lignified peltate trichome of durian. Each attempt is marked by an appressorium-like swelling. Scale bar = 20 μm.

Figure 3.2.5 A scanning electron micrograph of a *Phytophthora palmivora* sporangium which has germinated on the surface of a peltate trichome and grown over the edge to the epidermal surface of the durian fruit below. Scale bar = 10 μm.



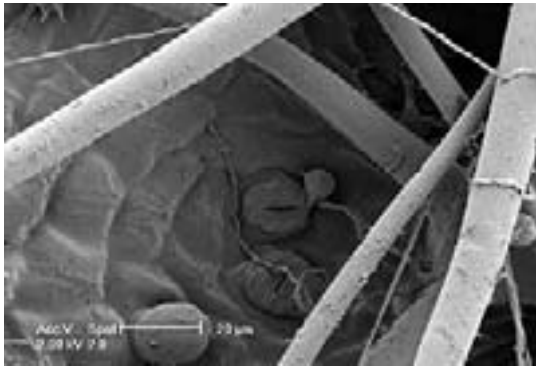


Figure 3.2.6 A germinated cyst of *Phytophthora palmivora* which has grown over the top of a stoma on the lower surface of a durian leaf, with no attempt at penetration. Note: glandular trichome (arrow). Scale bar = 20 μm .

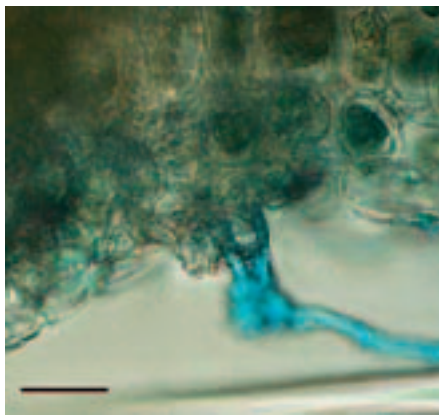


Figure 3.2.8 A light micrograph of a whole leaf mount showing penetration of a stoma on the lower surface of a durian leaf by *Phytophthora palmivora* (stained with lactophenol cotton blue). Scale bar = 25 μm .

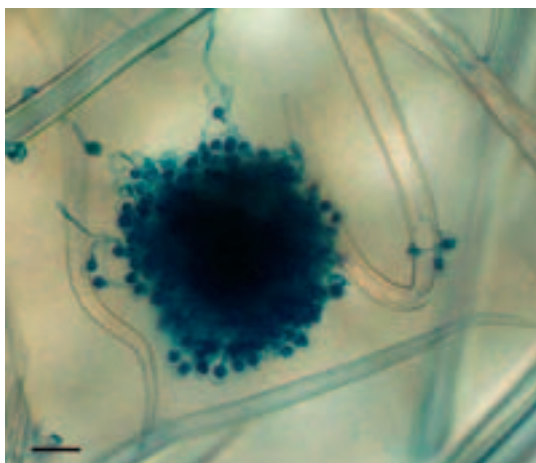


Figure 3.2.9 A light micrograph of a cleared whole leaf mount showing large numbers of *Phytophthora palmivora* zoospore cysts (stained with lactophenol cotton blue) preferentially attracted to, and germinated on, a fresh wound on the lower surface of a durian leaf. Scale bar = 25 μm .



Figure 3.2.7 An axillary shoot of durian. One stipule has been excised and placed to the right to show the newly formed and as yet still folded leaf (arrow) and a new generation of shoots within the fused stipules on the left. The external surface of the stipules, folded leaf and young bud are covered with peltate trichomes, but the inner side of the stipule (circle) is free of peltate trichomes. Bars on left = 1 mm spacings.

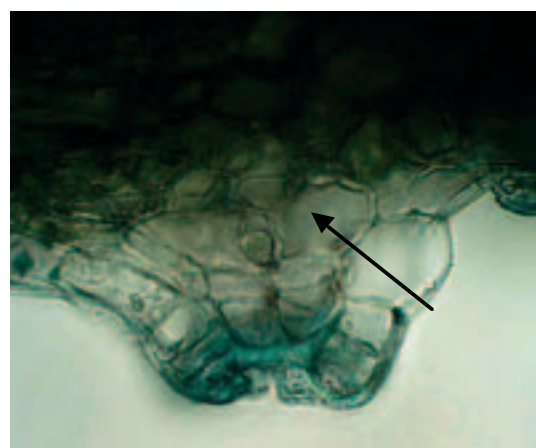


Figure 3.2.10 Deposition of suberin (blue/grey stain) 48 hours after the deliberate removal of a peltate trichome from a durian leaf. Suberin was detected with Sudan Black B and is deposited mainly in the cells at the point where the trichome was attached ($\times 400$).

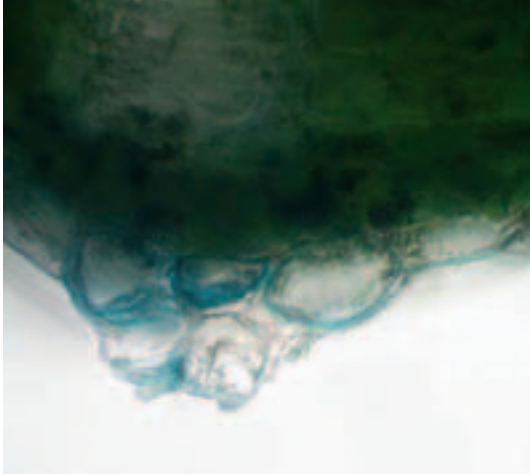


Figure 3.2.12 Deposition of callose (blue staining) 2 weeks after the deliberate removal of a peltate trichome from a durian leaf. Callose was detected with resorcinol blue and was deposited around the wall of cells throughout the trichome mound ($\times 400$).



Figure 3.2.15 A scanning electron micrograph of sporangia of *Phytophthora palmivora* produced as secondary inoculum and emerging above the peltate trichomes on a heavily infected durian fruit. Scale bar = 10 μm .

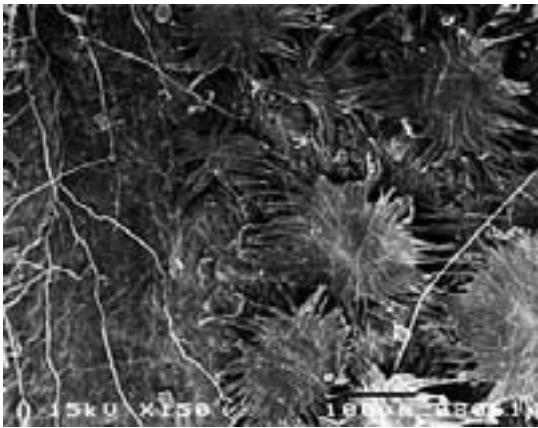


Figure 3.2.13 A scanning electron micrograph of zoospore cysts of *Phytophthora palmivora* that have bound and germinated in the trichome-free region between the spines on the durian fruit. Scale bar = 100 μm .

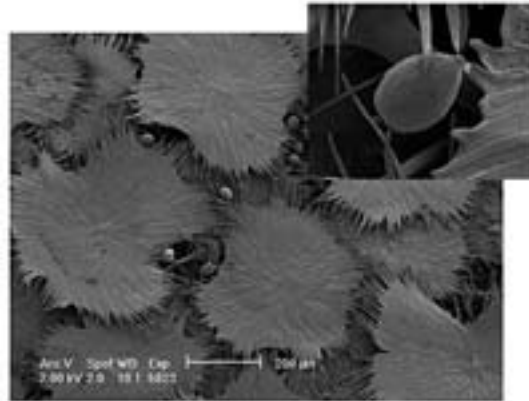


Figure 3.2.16 Sporangia of *Phytophthora palmivora* at the surface of a durian leaf where they can be readily distributed to the wider environment by water, insects and possibly wind. Sporangia have become detached from the hyphal body that has erupted through the surface of infected tissues beneath the trichomes. The inset picture is an enlargement of the sporangium within the square.

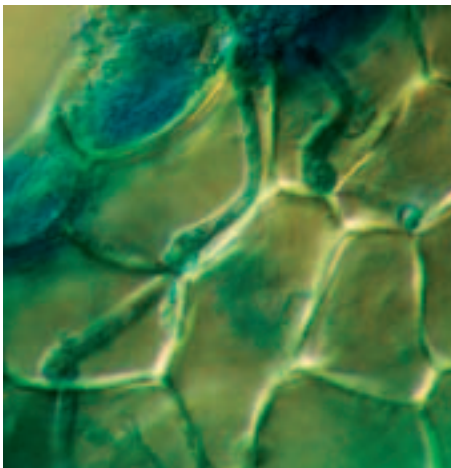


Figure 3.2.14 Differential interference contrast micrograph showing intracellular growth of *Phytophthora palmivora* in a durian leaf, stained with lactophenol cotton blue. Note the swelling of the hypha at the point of wall penetration indicating the direction of growth ($\times 400$).



Figure 3.2.17 A durian fruit infected with *Phytophthora palmivora*. The white bloom in the middle of the lesion is hyphae and sporangia that have formed on the surface of the lesion.

The Effect of Phosphonate on Symptom Development and the Activity of PAL in Durian

The treatment of durian seedlings with 1 g a.i. phosphonate led to significantly smaller lesions ($F_{1,8} = 8.14; p = 0.02$) when attached leaves were inoculated with *P. palmivora* (Figure 3.2.18). Within 9 days of inoculation, all leaves from the untreated trees were totally diseased or had abscised. In contrast, leaves from the phosphonate-treated seedlings were still attached 21 days after inoculation and lesion development was restricted (Figure 3.2.19).

No significant difference in lesion size between phosphonate-treated and untreated seedlings was observed when leaves were detached before inoculation (Figure 3.2.18), although lesions from the untreated seedlings were surrounded by a chlorotic halo not present in leaves from phosphonate-treated seedlings (Figure 3.2.20).

Activity of PAL significantly ($P < 0.05$) increased within 48 hours of inoculating detached leaves of durian with *P. palmivora* whether the leaves were from treated or untreated seedlings (Figure 3.2.21). The apparently higher levels of PAL activity in inoculated leaves from phosphonate-treated seedlings compared to leaves from untreated seedlings were not significant (Figure 3.2.21).

Discussion

Phytophthora palmivora is attracted to fresh wounds in durian which make them key infection courts. Taxis of zoospore/cysts was evident from the manner in which they amassed on the wound with germ-tubes aligned toward it, as *Phytophthora cinnamomi* propagules have been shown to do in zones of chemotaxis on roots (Hardham and Gubler 1990).

Natural wounds or those caused by pruning are considered key infection courts of *Phytophthora syringae* in apple (Sewell and Wilson 1964) and *P. citricola* in avocado (El-Hamalawi et al. 1995). Leaf scars have been identified in apple and peach as infection courts for *Nectria galligena* (Crowdy 1952) and *Leucostoma* spp. (Biggs 1997), respectively, and should be examined in durian as potential sites of ingress for *P. palmivora*, given the pathogen's attraction to fresh wounds and the potential for tree injury during typhoons.

We have shown that it takes 24–48 hours from the time of wounding for suberin, callose and lignin to become visually detectable in durian leaves, and this is likely to take longer in woody organs. We have also shown that under optimal conditions, the pathogen infects, ramifies in tissue and reproduces very rapidly and would thus be able to produce many generations of propagules in the time taken to wound healing. Consequently, care should be taken to prune durian when weather conditions are not conducive to disease, and treatment of cut surfaces should be considered.

Stomata have been identified in a previous study as infection courts of *P. palmivora* in cocoa pods (Iwara et al. 1997). While this is also the case in durian, there appeared to be no preferential attraction and stomata were penetrated by a single hypha.

Trichomes are a common feature of species in the Bombacaceae family (Metcalf and Chalk 1950), but are more complex in *Durio* than in other genera (Baas 1972). The absence of the overlapping peltate trichomes from the trough between the spines on the durian fruit make it particularly vulnerable to infection. However, trichomes on other organs such as the young stem, petiole and the underside of the leaf do not always protect from infection, as the pathogen can grow extensively before penetration (presumably utilising an endogenous nutrient supply) with the potential to grow over the side of the trichome to the underlying epidermis, including the stomata.

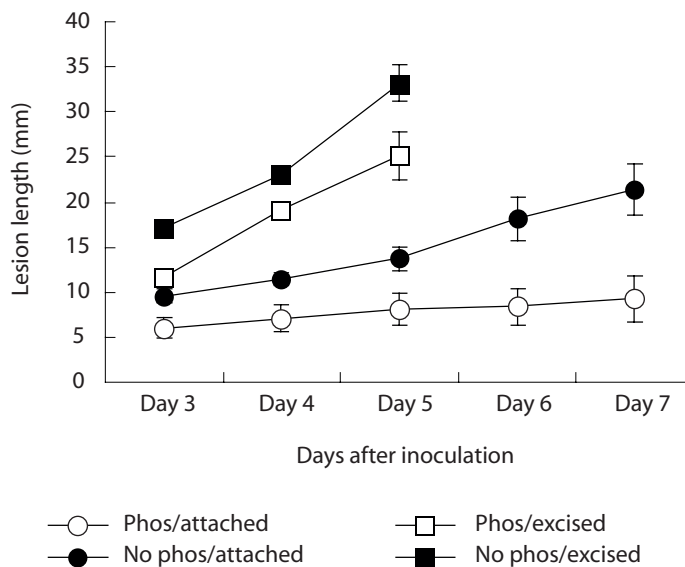


Figure 3.2.18 Mean lesion length resulting from inoculation with *Phytophthora palmivora* of leaves from phosphonate-treated or untreated durian seedlings when leaves were either excised before inoculation or inoculated while still attached to the seedling.

Although fresh wounds and stomata are considered key infection courts, *P. palmivora* is capable of direct penetration of leaf and fruit tissues. However, direct penetration by hyphae was observed rarely, compared to extremely common stomatal infections. It is unlikely that direct penetration would cause significant disease in healthy tissues but this mode of infection probably becomes increasingly important in ripening fruit.

Opportunistic infections such as stomatal and direct infections are made possible by the ability of *P. palmivora* zoospores to randomly bind and germinate on the rough surface of durian produced by trichomes. Randomly bound spores of *P. palmivora* attempted to penetrate the heavily lignified trichomes at 'appressoria-like' swellings, but were apparently unsuccessful as hypha emerged from the swelling and resumed growth across the tissue. According to Emmett and Parbery (1975), the definition of a 'true' appressorium is any structure that adheres to the host surface, with the ability to germinate and penetrate through the production of an infection peg.

'Appressoria-like' swellings were also produced by *P. cinnamomi* with apparently unsuccessful attempts to penetrate phellem cells of *Eucalyptus marginata* (O'Gara 1998). These types of structures have been observed in other phytophthora pathosystems (Beagle-Ristaino and Rissler 1983; Swiecki and McDonald 1988). Hardham (2001) suggests they are often associated with attempted penetration of

relatively resistant tissues — for example, they occur when *P. cinnamomi* penetrates the periclinal root wall of onion but not the anticlinal wall.

Phytophthora cinnamomi is preferentially attracted to the axillary shoots of jarrah and they are considered key infection courts (O'Gara 1998). In contrast, *P. palmivora* showed no attraction to the axillary shoots of durian. However, the emerging shoots of durian are well protected by trichome-covered stipules and by the time of bud opening, the lower surface of the leaf has a trichome covering, while emerging jarrah shoots are devoid of stipules, trichomes and indeed the leaf cuticle is either extremely thin or absent (O'Gara 1998).



Figure 3.2.19 A highly restricted lesion on a leaf from a phosphonate-treated durian seedling, 21 days after inoculation with *Phytophthora palmivora*. Scale bar = 1 cm.

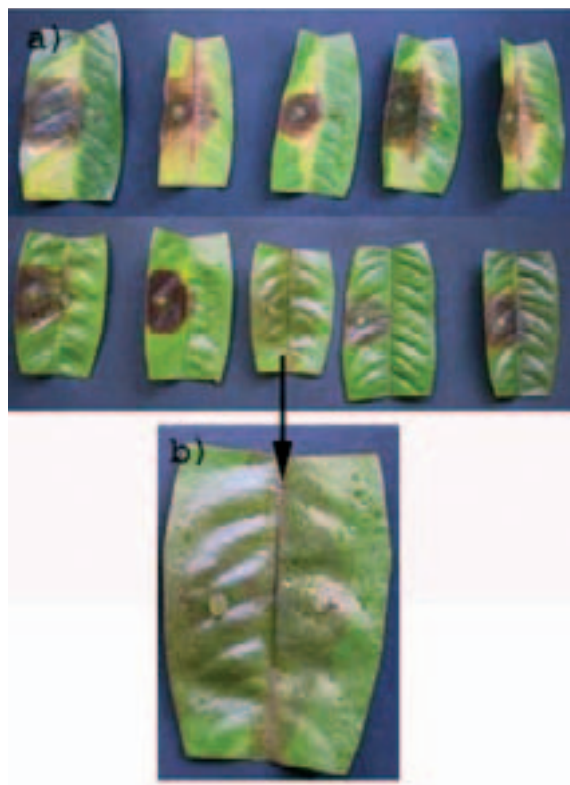


Figure 3.2.20 Lesion development in excised durian leaves 4 days after inoculation with *Phytophthora palmivora* on the left side of the mid-vein (a). Although there was no difference in size, lesions from untreated seedlings (top row) were surrounded by a chlorotic halo while leaves from phosphonate-treated seedlings (bottom row) were not. Note the variability in lesion appearance between leaves from the phosphonate-treated (bottom row) seedlings. There appears to be no lesion on the middle leaf, but the enlarged photo (b) shows that it has a water-soaked, pale lesion with diffuse boundary which has almost entirely covered the leaf.

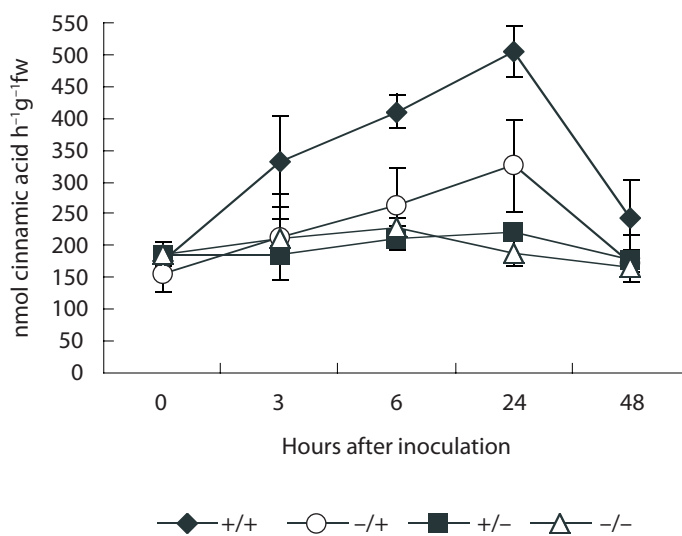


Figure 3.2.21 Phenylalanine ammonia-lyase (PAL) activity in leaves from phosphonate-treated and untreated seedlings of durian that were inoculated with *Phytophthora palmivora* after excision. Treatment combinations include: (+/+) = phosphonate and *P. palmivora*; (-/+) no phosphonate and *P. palmivora*; (+/-) = phosphonate and no *P. palmivora*; and (-/-) = no phosphonate and no *P. palmivora*.

It was originally hoped that an excised leaf bioassay, such as that developed to assess natural resistance to *P. palmivora* (see Chapter 8.3), could also be used to estimate phosphonate concentrations in durian tissues. However, the effect of phosphonate could not be demonstrated in leaves that were excised before inoculation, but was readily demonstrated

when attached leaves were inoculated, through highly restricted symptom development. It appears that the excision of leaves may interrupt the phenylpropanoid pathway signalling, as there was no significant difference in lesion development or PAL activity in excised inoculated leaves from untreated or treated seedlings.

The information gathered in the current study, coupled with field observations, improves our understanding of infection biology in durian orchards. Under mild weather conditions, the pathogen is at relatively low levels and individual spores infect individual stomata (if they can first negotiate the trichome armour), or isolated propagules directly penetrate through the epidermis. However, the host can resist these limited attacks and disease levels remain low. When conditions are extreme though, such as during typhoons, cyclones, or when 5–6 days of continuous rainfall occur, as can happen during the monsoon, the inoculum levels increase rapidly and tree injury provides numerous infection courts to which large numbers of zoospore/cysts are attracted. The pathogen reproduces faster than the infection-court-wounds heal. The synergism of the amassed spores enables the pathogen to overcome the host's capacity to impede the growth of a single hypha (Hinch et al. 1995). The pathogen ramifies in the infected tissues, and erupts through the surface of the organ, releasing more propagules to fuel the epidemic. The 'multi-cyclic' nature of the infection biology just described in durian is similar to that of *P. palmivora* in cocoa, which has been called a 'compound continuous interest' disease (MacKenzie et al. 1983; see also Chapter 6.2).

In conclusion, the current study has provided new information on the infection biology of *P. palmivora* in durian. While the information presented in this paper is extremely valuable from a purely academic perspective, it has also assisted in the understanding of the disease aetiology and epidemiology and was a key component in the formulation of integrated disease management options for phytophthora diseases in durian. However, there is much more that could be learnt about the host/pathogen interaction at a cellular and molecular level, which would enable fine-tuning of current recommendations.

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3.3 Morphological and Host Range Variability in *Phytophthora palmivora* from Durian in Thailand

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Abstract

Comparative morphological, physiological, and pathological tests showed that all isolates of *Phytophthora* isolated from durian orchards in Thailand are *Phytophthora palmivora*. Sporangia of 26 isolates were caducous with short pedicels (2.8–4.2 µm), but were variable in shape and size. The cultures produce ovoid, ellipsoid, obpyriform, ovoid-obpyriform, and spherical sporangia, average 35 to 90 µm in length and 22 to 62 µm in breadth, and have a length/breadth ratio of 1.6 to 2.0. The *P. palmivora* isolates also caused brown lesions on black pepper and rubber.

Introduction

The oomycete *Phytophthora palmivora* Butl. is a serious pathogen of durian, causing trunk canker, fruit and root rot in Thailand. *P. palmivora* infects durian fruit at the ripening stage and causes a soft brown lesion on the skin (Lim 1990; Pongpisutta and Sangchote 1994). Many durian plantation areas in the south and east of Thailand are close to rubber and black pepper plantation areas, and some growers plant these trees as intercrops in durian orchards. Orellana (1959) studied the pathogenicity of *P. palmivora* isolated from cocoa in which it was causing black pod rot, and from rubber a *Phytophthora* species causing fruit rot and defoliation. When unwounded leaves, petioles and terminal buds of young rubber seedlings were inoculated with *P. palmivora* from cocoa, disease occurred within 7–8 days, but isolates of the *Phytophthora* species from rubber did not cause symptom development after inoculation on comparable parts of cocoa plants under the same conditions in the greenhouse and laboratory. Since at least seven different *Phytophthora* species (Erwin and Ribeiro 1996) have the ability to cause disease on rubber, the identification of this particular isolate remains unknown. Tsao and Tummakate (1977)

collected a number of *Phytophthora* isolates causing foot and root rot disease from a black pepper plantation Amphur Palien in Trang province in southern Thailand. The Thai black pepper isolates produced narrow, ellipsoid, obovoid, pyriform sporangia with a tapered base, instead of the rounded or hemispherical base common in *P. palmivora*. However, using Tsao (1991), these isolates would probably at present be identified as *P. capsici* (= *P. palmivora* MF4).

The aims of the research described in this paper were: (i) to determine the *Phytophthora* species occurring on durian in Thailand; (ii) to determine the range of morphological characteristics from *Phytophthora* isolates obtained; and (iii) to investigate the host range of *Phytophthora* from durian. This information may be useful for identifying *Phytophthora* species, for choosing planting sites and for determining intercropping practices. Since *P. palmivora* has a wide host range including a number of important food crops in the tropics, recommendations for intercropping need to be backed up by long-term field experiments involving intercropping of hosts susceptible to *P. palmivora* in this area.

Materials and Methods

Isolation of the pathogen

Isolates of *P. palmivora* were obtained from soils and diseased leaves, branches, and stems of durian from

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different locations in eastern Thailand (Table 3.3.1). Isolations from durian were assessed using a tissue transplanting method. Tissue was cleaned under running tap water and plated on selective agar containing benomyl (10 ppm), nystatin (50 ppm), pentachloronitrobenzene (PCNB) (25 ppm), ampicillin (500 ppm), rifampicin (10 ppm) and hymexazol (45 ppm). For soil samples, a baiting technique was used. Small pieces of fresh durian leaves were exposed to soil for 2 days before placing on fresh selective agar.

Morphological characteristics

Colony characteristics were assessed on potato dextrose agar (PDA) after incubation at 25°C under near ultraviolet (UV) light for 5 days.

Phytophthora cultures were grown on carrot agar (CA) and incubated at 25°C under near UV for 7 days. Morphological characteristics of the asexual structure assessed included sporangia morphology (shape, size and length–breadth ratio), presence of papilla, caducity and chlamydospore production. These characters were determined by light microscopy of lactophenol-mounted slides.

Growth temperatures

Small discs of agar were cut from all isolates using a 5 mm cork borer, then placed on CA and incubated

at 10, 15, 20, 25, 30, 35, and 37°C for 7 days, after which the colony diameter was measured.

Pathogenicity test

Isolates of *Phytophthora* from durian were tested for pathogenicity against durian, rubber and black pepper by artificial inoculation of wounded leaves. A needle was used to wound leaves before placing 5 mm mycelial discs from each isolate upon separate wounds. The inoculated leaves were incubated in plastic bags for 24 hours. Pathogenicity was measured as the length–breadth ratio of brown lesions 5 days after inoculation.

Results

Phytophthora cultures obtained

Twenty- six isolates were recovered from infected parts of durian such as fruit, stem, leaf and branch (Table 3.3.1). These morphological characteristics of the isolates were compared to the *P. palmivora* description in Erwin and Ribeiro (1996).

Morphological characteristics

Most cultures grew on PDA with a stellate pattern, except for P09, P27, P31, and P33, which were radiate, irregular and slightly fluffy, slightly petallate and stoloniferous colonies, respectively (Table 3.3.2 and Figure 3.3.1).

Table 3.3.1 Sources of *Phytophthora* isolated from durian in Thailand.

Isolate no.	Host tissue	Location	District	Province	Year of collection
P01	fruit	Toong Benja,	Tamai	Chantraburi	1997
P03	fruit	Toong Benja	Tamai	Chantraburi	1997
P04	fruit	Sagthai	Tamai	Chantraburi	1997
P05	fruit	Khao Baisri	Tamai	Chantraburi	1997
P07	fruit	Khao Baisri	Tamai	Chantraburi	1997
P09	fruit	Khao Baisri	Tamai	Chantraburi	1997
P10	fruit	Khao Baisri	Tamai	Chantraburi	1997
P12	fruit	Toong Benja	Tamai	Chantraburi	1998
P14	fruit	Toong Benja	Tamai	Chantraburi	1998
P17	soil	Sagthai	Tamai	Chantraburi	1998
P19	stem	Ta Chang	Mueng	Chantraburi	1998
P21	fruit	Mueng	Mueng	Prachin Buri	1998
P22	stem	Mueng	Mueng	Prachin Buri	1998
P23	stem	Mueng	Mueng	Chantraburi	1999
P25	leaf	Praneet	Khao Saming	Trat	1999
P26	branch	Praneet	Khao Saming	Trat	1999
P27	leaf	Khao Saming	Khao Saming	Trat	1999
P29	stem	Sagthai, Tamai	Tamai	Chantraburi	1999
P31	branch	Toong Kwai Hin	Klang	Rayong	1999
P32	leaf	Toong Kwai Hin	Klang	Rayong	1999
P33	branch	Toong Kwai Hin	Klang	Rayong	2000
P35	fruit	Ta Chang	Mueng	Chantraburi	2000
P36	fruit	Ta Chang	Mueng	Chantraburi	2000
P37	fruit	Khao Saming	Khao Saming	Trat	2000
P38	stem	Lang Suan	Lang Suan	Chumphon	2000
P39	branch	Lang Suan	Lang Suan	Chumphon	2000

Table 3.3.2 Asexual characteristics of *Phytophthora* from durian in Thailand

Isolate no.	Chlamydospores			Sporangia				
	Colony pattern	Type	Average diameter (µm)	Pedicel length (µm)	Length (µm)	Breadth (µm)	L:B ratio	Shape
P32	stellate	terminal	36	3.9	48-72	28-38	1.8	Ovoid-obpyriform, Ellipsoid
P25	stellate	terminal & intercalary	34	3.4	48-72	28-40	2.0	Ovoid-obpyriform, Ellipsoid, Obpyriform
P31	slightly petallate	terminal	34	3.0	48-72	25-38	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform
P35	stellate	terminal	37	4.1	50-75	28-42	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform
P39	stellate	terminal	33	3.0	48-80	28-42	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform
P07	stellate	terminal	34	2.9	43-80	28-40	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform, Ovoid
P01	stellate	terminal & intercalary	36	4.0	50-73	28-45	1.9	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P04	stellate	terminal	36	4.2	38-75	25-35	2.0	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P09	radiate	terminal	34	2.8	40-65	28-45	1.6	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P10	stellate	terminal	32	2.9	41-65	29-44	1.7	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P14	stellate	terminal	37	2.8	48-78	28-42	1.9	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P19	stellate	terminal & intercalary	36	3.5	48-70	28-48	1.7	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P22	slightly stellate	terminal	38	4.4	50-70	28-42	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P23	stellate	terminal	34	3.5	45-72	25-42	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P27	irregular	terminal	34	3.3	45-78	25-40	1.9	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P29	stellate	terminal	34	3.4	40-78	28-40	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P36	stellate	terminal	34	3.6	42-85	22-40	1.9	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P38	stellate	terminal	39	3.3	45-90	28-50	1.8	Ovoid-obpyriform, Ellipsoid, Obpyriform, Spherical
P12	stellate	terminal	38	3.2	45-72	28-45	1.6	Ovoid-obpyriform, Obpyriform, Spherical
P17	stellate	terminal & intercalary	32	3.4	42-75	25-38	1.8	Ovoid-obpyriform, Obpyriform, Spherical
P21	stellate	terminal	36	3.0	48-82	28-42	1.8	Ovoid-obpyriform, Obpyriform, Spherical
P33	stoloniferous	terminal	30	3.9	35-65	22-38	1.7	Ovoid-obpyriform, Obpyriform, Spherical
P37	stellate	terminal	34	3.4	42-72	28-42	1.7	Ovoid-obpyriform, Obpyriform, Ovoid, Spherical,
P26	stellate	terminal	36	3.2	52-78	25-38	2.0	Ovoid, Ellipsoid, Obpyriform
P05	stellate	terminal	35	3.5	48-78	28-45	1.8	Ovoid, Ellipsoid, Obpyriform, Spherical
P03	stellate	terminal & intercalary	35	3.9	39-74	26-35	1.9	Ovoid, Obpyriform, Spherical

Twenty-six isolates produced ovoid, ellipsoid, obpyriform, ovoid-obpyriform, and spherical sporangia (Figure 3.3.2). The sporangia were caducous, with a short pedicel (2.8–4.2 μm) and conspicuously papillate. A few isolates showed bipapillate sporangia. The sporangia were variable in size (Table 3.3.2), averaging 35–90 μm in length and 22–62 μm in breadth, with a length–breadth ratio of 1.6–2.0. All isolates produced globose chlamydospores, which were terminal and intercalary in the mycelium. The average diameter of chlamydospores was 30–39 μm . Based on these morphological characteristics, all isolates belonged to *P. palmivora*.

Growth temperatures

All isolates grew at 10°C, with colony diameters less than 6 mm after 7 days. The optimum temperature was 25°C, with diameters of most isolates about 80–90 mm, and the maximum temperature 35°C, with

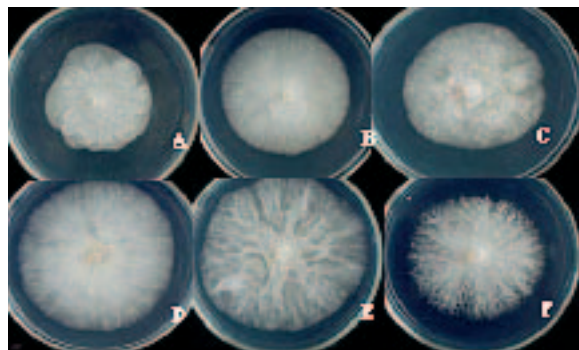


Figure 3.3.1 Colony types of *Phytophthora* isolated from durian on PDA after 5 days incubation: A, stellate pattern; B, slightly stellate pattern; C, slightly petallate pattern; D, radiate pattern; E, irregular pattern and slightly fluffy; F, stoloniferous pattern.

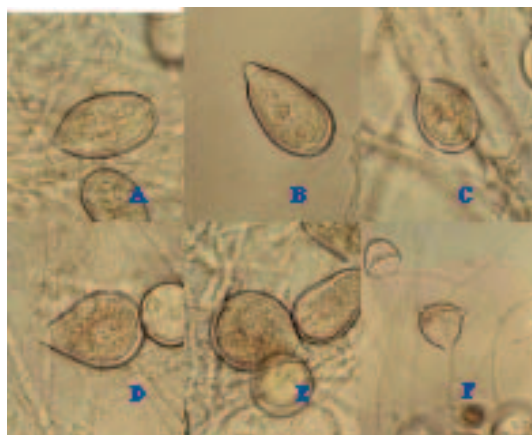


Figure 3.3.2 Morphology of sporangia: A, ellipsoid; B, obpyriform; C, ovoid; D, ovoid-obpyriform; E, spherical; F, bipapillate sporangium.

growth diameters around 55–72 mm. The exception was isolate P14, which could not grow at that temperature. No isolates grew at 37°C. Cardinal temperatures were thus minimum < 10°C, optimum 25°C and maximum 35°C.

Pathogenicity test

All *Phytophthora* isolates from durian were successful in infecting wounded leaves of durian, black pepper and rubber. The isolates produced lesions of variable size on different host plants. They caused large lesions on durian leaves, and brown lesions on black pepper and rubber. Most of the isolates from durian were more aggressive on rubber than on black pepper (Table 3.3.3).

Table 3.3.3 Results of pathogenicity tests of *Phytophthora* isolates from durian on wounded leaves of durian, black pepper and rubber.

Isolate no.	Diameter of disease lesion (mm)		
	Durian	Black pepper	Rubber
P01	14.9	9.7	7.5
P03	10.9	5.3	5.7
P04	17.2	5.3	7.8
P05	14.1	4.7	14.2
P07	14.7	9.1	8.5
P09	16.0	9.7	10.4
P10	14.5	6.8	9.6
P12	14.2	0	9.0
P14	16.1	11.4	10.4
P17	11.4	8.3	10.5
P19	12.8	6.1	8.7
P21	17.0	6.3	8.9
P22	21.1	9.1	9.2
P23	13.7	9.4	12.9
P25	9.0	8.9	16.0
P26	10.5	8.0	9.4
P27	10.2	9.8	12.2
P29	13.2	8.1	7.8
P31	9.5	7.9	9.0
P32	11.3	0	10.0
P33	13.0	0	7.5
P35	13.3	7.5	7.4
P36	12.9	8.2	9.9
P37	18.4	7.3	8.7
P38	15.7	9.1	8.5
P39	10.6	9.8	14.3

Discussion

Several researchers have described the features of *P. palmivora* that distinguish it from other heterothallic species with conspicuous papillate sporangia. The sporangia are variable in shape, depending on isolate, mostly elliptical to ovoid, and prominently papillate. They are caducous with a

short pedicel (< 5 µm), and are variable in size but average 40 to 60 µm in length and 25 to 35 µm in breadth, with length–breadth ratio of 1.4 to 2.0 µm (Ho 1990; Erwin and Ribeiro 1996). On a few occasions we also observed spherical bipapillate sporangia

Many reports have shown that *P. palmivora* produces globose chlamydospores. Chlamydospore diameters have been reported to measure 32 to 42 µm (Holliday 1980), averaging 33 µm (Waterhouse 1974), 36 µm (Ashby 1929) and 36.2±9.6 µm (Mchau and Coffey 1994).

Most isolates produced stellate colony types. Waterhouse et al. (1983) reported that *P. palmivora* colonies were stellate. In our study, only one isolate (P33) showed a stoloniferous growth pattern, but other morphological characters confirmed the identity of this isolate as *P. palmivora*. The data produced on the isolates in our study fall within this range, confirming the species identity.

Waterhouse (1974) studied the effect of temperature on the growth of *P. palmivora* and reported the minimum temperature as 11°C, the optimum as 27.5–30°C, and the maximum as near 35°C.

Pathogenicity tests by many researchers have shown that *P. palmivora* isolates that cause black stripe and patch canker of rubber, and leaf and collar rot of black pepper in Southeast Asia, can also cause patch canker of durian (Belgrave and Norris 1917; Navaratnam 1966; Tsao and Tummakate 1977; Suzui et al. 1979). *Phytophthora nicotianae* has also been reported as infecting durian, causing patch canker, and fruit, crown, foot, and root rot of black pepper, especially in Malaysia and Thailand (Liu 1977; Suzui et al. 1979). However, all isolates collected in this study were identified as *P. palmivora*.

The results of earlier research on the causes of leaf and collar rot of black pepper have often given *P. palmivora* as the likely causative agent. However, a more recent reclassification of pepper isolates of *P. palmivora* MF4 as *P. capsici* indicate that further taxonomic and genetic studies are needed to more clearly define the boundaries between these *Phytophthora* species (Tsao and Alizadeh 1988; Tsao 1991).

This study revealed that there is variation in the *P. palmivora* population obtained from durian in Thailand. This variation within as well as between species makes identification of these species more difficult. Some of the phenotypic variation observed may also be due to environmental factors such as the media and temperature used to culture *Phytophthora*

species. More accurate species identification may be achieved through the use of molecular-based identification methods. This is especially important considering the wide range of *Phytophthora* species occurring in the tropics.

With intercropping gaining in popularity it is important to know the species of *Phytophthora* involved in disease. Many host plants susceptible to *P. palmivora* are grown throughout Southeast Asia. Intercropping of hosts susceptible to the same pathogens may give rise to an increased build-up of inoculum and thus be responsible for disease problems more severe than those encountered in monoculture situations.

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4

Occurrence of *Phytophthora* in Southeast Asia



4.1 Phytophthora Diseases in Malaysia

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Abstract

This chapter provides a historical overview of the *Phytophthora* species found in Malaysia and details on the occurrence, impact and control of the main phytophthora diseases affecting Malaysia's major agricultural crops: rubber, cocoa, durian and pepper.

Introduction

Malaysia is made up of two geographical regions, namely Peninsular Malaysia on the southeastern tip of mainland Asia, and the states of Sabah and Sarawak on the island of Borneo. The South China Sea separates the two regions. Situated just north of the equator, the climate is typically hot and humid tropical. It has an annual rainfall of 2000–4000 mm, falling in 150 to over 200 days per annum. For example, the foothills of the Cameron Highlands in Peninsular Malaysia and Kuching in Sarawak experience about 250 rainy days per annum. Average daily temperature under shade ranges from 23 to 29°C with relative humidity in the range 70–90%. These climatic conditions are ideal for year-round cultivation of tropical crops. They are also excellent for the development and spread of tropical plant diseases.

Agriculture in Malaysia is dominated by mega plantations, with extensive planting of monocultures of rubber, oil palm, cocoa and coconut. This export-oriented agricultural system was first introduced into the country in the late 1800s. Phytophthora diseases are common on rubber and cocoa. There are no reports of phytophthora on oil palm. The incidence of phytophthora on coconut is sporadic, although *Phytophthora nicotianae* (syn. *P. parasitica*) has occasionally been isolated from infected palms.

Among non-plantation crops, durian (*Durio zibethinus* L.) is, next to rice, the most important crop, in terms of area planted. It is also the most popular fruit in Southeast Asia, the centre of biodiversity for *Durio* species. One of the most important factors limiting the planting and production of durian is trunk and root rot caused by *Phytophthora palmivora*.

About 60,000 farm families, mostly from Sarawak, are involved in pepper cultivation. This makes pepper the most important cash crop in Sarawak. Foot rot caused by *P. capsici* is the most serious disease limiting the successful cultivation of the crop. Johor in Peninsular Malaysia was at one time an important centre for pepper production, but most of the farms have now been converted to non-agricultural uses.

Research into the genus *Phytophthora* in the country started about 80 years ago and is centred mostly on rubber, cocoa, durian and pepper, which together occupy an area of about 1.6 million ha. Some phytophthora research, mostly limited to identification and control, had also been carried out on crops such as citrus, papaya, guava, passionfruit, jackfruit, roselle, tomato, potato, yam and orchids.

Phytophthora Species and Their Recorded Hosts

Plant pathological work in the early 1900s was focused mainly on rubber and spices. The person who contributed most to the understanding of phytophthora in the early 1900s was A. Thompson. In 1925, he recorded a *Phytophthora* species that caused patch canker of rubber (*Hevea brasiliensis* (H.B.K.) Mull. Arg.) (Thompson 1925). A year later,

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he recorded another *Phytophthora* species on betel vine (*Piper betle* L.), which he described as either *P. parasitica* or *P. colocasiae* (Thompson 1926). In his preliminary report on *Phytophthora* species in Malaysia, Thompson (1928) noted that there were only two recorded species. In 1929, he recorded three more species, namely *P. palmivora*, *P. heveae*, and *P. meadii* on rubber (Thompson 1929).

Sudden death of pepper (*Piper nigrum* L.), possibly caused by phytophthora, was first reported by Holl (1929) in Sarawak. Thompson (1941) and Holliday and Mowat (1957) isolated a species of *Phytophthora* from infected pepper vines. Several years later, Holliday and Mowat (1963) identified the fungus as an atypical strain of *P. palmivora*. The first report of the disease in Johor was by Loh (1970).

Sharples (1930) recorded *P. nicotianae* (described as *P. parasitica*) on *Hibiscus sabdariffa* L., a plant grown for its fibre at that time. In the early 1990s, this crop was reintroduced for juice extraction on a commercial scale in the east coast of Peninsular Malaysia. On mineral soil, *P. nicotianae*, causing sudden wilt symptoms, was frequently isolated from infected roots and collars of the plant, especially during the wet monsoon months from October to January (B.S. Lee, unpublished data). Interestingly, the crop was free of phytophthora symptoms when planted on irrigated sandy soil.

Thompson (1934a) described for the first time the occurrence of *P. palmivora* as the causal agent of patch canker on durian (*Durio zibethinus*) in Penang. He observed that the disease had been present in the locality for at least the previous 10 years, and that it had killed many mature trees. Subsequently, the disease was extensively studied by Chan and Lim (1987), Lee (1999), Lee and Varghese (1974), Lim and Chan (1986), Lim and Yassin (1985), Navaratnam (1966), and Tai (1971). Although durian trees in Penang remain badly affected by phytophthora (Hashim et al. 1991), the state has remained famous for its unique varieties of durian.

Thompson (1934b) reported the occurrence of *Phytophthora infestans* on potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) in the Cameron Highlands, while *P. parasitica* was recorded on tomato several years later (McIntosh 1951). Leaf blight and fruit rot on tomato, caused by *P. infestans*, are still limiting factors to about 700 ha of tomato in the Cameron Highlands. Tolerant varieties, fungicidal sprays, and planting of tomato under plastic rain shelter have reduced the problem.

Thompson (1940) identified *P. palmivora* as the causal agent of root and collar rot on papaya (*Carica*

papaya L.). Fruit rot caused by *P. palmivora* is common under wet weather conditions. *P. parasitica* is another species commonly isolated from infected fruit, collar and roots of papaya (Singh 1973; B.S. Lee, unpublished data). In general, phytophthora is not a serious problem on papaya, except when replanting is practised (Lee and Chan 1980). The disease is often localised and occurs in situations where soil drainage is poor (Lim and Yaakob 1989).

Cocoa (*Theobroma cacao* L.) was first introduced in the 1940s, but it was not until the 1950s that it was successfully planted on a commercial scale. Barcroft (1961) reported the first incidence of black pod caused by *P. palmivora* in the country. Ten years later, Chee and Phillips (1971) reported the occurrence of stem canker.

Wong and Varghese (1966) spent several years researching on the biology, ecology and control of foot and root rot of citrus. They attributed the diseases to *P. nicotianae*. Chee (1969b) studied the sudden outbreak of abnormal leaf fall of *Hevea* rubber in the northern states of Perlis, Kedah and Perak and described the pathogen as a new species, which he named as *P. botryosa* Chee. An extensive search for *Phytophthora* in the early 1970s resulted in the isolation of *P. capsici* from bell pepper (*Capsicum annum* var. *grossum* L.), *P. heveae* from cocoa, and *P. nicotianae* from brinjal (*Solanum melongena* L.) and strawberry (*Fragaria* sp.) (Lee 1972).

Singh (1973), in his compilation of plant diseases, made several additions to the increasing list of *Phytophthora* species in the country: *P. colocasiae* on yam (*Colocasia esculenta* Schott.) and *Piper betle*; *P. palmivora* on Vanda orchids; *Phytophthora* sp. on avocado (*Persea gratissima*), *P. nicotianae* on *Salvia splendens* and *P. nicotianae* on *Vinca rosea*.

Phytophthora cinnamomi was reported to cause root rot and dieback of quinine tree (*Cinchona ledgeriana* Moens and *C. succirubra* Pav. Ex. Klotzsch) in the Cameron Highlands (Thompson 1940). Lee (1974) isolated *P. cinnamomi* from infected roots of cloves (*Eugenia aromatica* Baill.). Kueh and Khew (1982) isolated *P. meadii* from roots of *Piper betle*. Chan and Lim (1987) reported *P. nicotianae* as the causal agent of leaf blight of guava (*Psidium guajava* L.). This was the last published record of a new incidence of phytophthora on any crop, although species of *Phytophthora* were isolated from various hosts from time to time (*P. nicotianae* from passionfruit and orchid, and *P. citrophthora* from jackfruit) (B.S. Lee, unpublished data).

Table 4.1.1 summarises this section.

Phytophthora Diseases of Economic Importance

Rubber

The earliest plantation crop in Malaysia was *Hevea* rubber. A number of seedlings from Brazil were sent to Kew Garden, then to the Botanical Garden of Singapore in 1877. That same year, some plants were sent to Malaysia. These few plants became the progenitors of all the large rubber plantings in Southeast Asia.

Total area planted with rubber has steadily declined from 1.69 million ha in 1995 to 1.43 million ha in 2000 (Table 4.1.2). This is expected to shrink further before stabilising at about 1.1 million hectares. The declining trend is due to the decline in the prices of primary commodities and the acute shortage of plantation labour. Despite the reduction, rubber cultivation will remain an important element in the Malaysian economy.

Most of the studies on the biology and control of phytophthora on rubber were done in the 1960s and 1970s (e.g. Chee 1968a,b, c; 1969a,b; 1970; 1971a,b; Lim and Abdul Aziz 1978; Tan et al. 1977; Tan 1979). In general, the stem, shoot, leaf and pod of the tree are attacked by two *Phytophthora* species, *P. palmivora* and *P. botryosa*. *P. palmivora* causes black stripe of the tapping panel and patch canker on the untapped bark, pod rot and leaf fall. On the other hand, *P. botryosa* is the main cause of leaf fall and pod rot diseases, although it may also cause black

stripe under conditions favourable to it. Leaf fall and black stripe are important diseases during the rainy seasons from July to October in the northern states of Perlis, Kedah and Perak, and from October to January in Kelantan. Most of the research has been centred on black stripe and leaf fall as they occurred more frequently than other phytophthora diseases (Tan 1979). Pathogenicity studies indicated that *P. palmivora* and *P. botryosa* from rubber were capable of infecting other crops including cocoa, durian, pepper, mango, citrus and orchid (Chee and Hashim 1971). No *Phytophthora* species has been recorded on rubber roots, although rubber root diseases caused by other groups of fungi are major constraints to the rubber industry.

Traditional methods of controlling rubber diseases rely heavily on the use of chemicals. Against leaf diseases, the application of fungicides on mature rubber trees is difficult due to the height of the trees, unsuitable terrain, poor accessibility and uneconomic plot size. Adequate control of black stripe is achieved by early detection and application of fungicides such as oxadixyl, metalaxyl and folpet. Against leaf fall, a pre-monsoon thermal fogging of copper-in-oil at 1.2 kg/ha has proven effective in controlling the disease (Lim 1982). Excellent control of leaf fall was also achieved by trunk injection with neutralised phosphorous acid (Lim and Lee 1990). Direct injection into the basal portion of the stem is easy and it dispenses with repeated rounds of expensive ground or aerial spraying. It also overcomes the problem with height of the rubber

Table 4.1.1 Host list of *Phytophthora* species isolated in Malaysia since 1925.

Species	Host	Collector
<i>Phytophthora</i> sp.	Rubber	Thompson (1925)
<i>P. colocasiae?</i>	Betel vine	Thompson (1926)
<i>P. palmivora</i>	Rubber	Thompson (1929)
<i>P. heveae</i>	Rubber	Thompson (1929)
<i>P. meadii</i>	Rubber	Thompson (1929)
<i>Phytophthora</i> sp.	Pepper	Holl (1929)
<i>P. nicotianae</i> (<i>P. parasitica</i>)	Roselle	Sharples (1930)
<i>P. palmivora</i>	Durian	Thompson (1934a)
<i>P. infestans</i>	Potato, tomato	Thompson (1934b)
<i>P. cinnamomi</i>	Quinine	Thompson (1940)
<i>P. palmivora</i>	Papaya	Thompson (1940)
<i>P. capsici</i> (<i>P. palmivora</i> atypical)	Pepper	Holliday and Mowat (1963)
<i>P. palmivora</i>	Cocoa	Barcroft (1961)
<i>P. nicotianae</i>	Citrus	Wong and Varghese (1966)
<i>P. botryosa</i>	Rubber	Chee (1969b)
<i>P. capsici</i>	Bell pepper	Lee (1972)
<i>P. heveae</i>	Cocoa	Lee (1972)
<i>P. nicotianae</i>	Brinjal, strawberry	Lee (1972)
<i>P. nicotianae</i>	Papaya	Singh (1973)
<i>P. colocasiae</i>	Yam	Singh (1973)

trees, the difficult terrain or high equipment, labour and chemical costs.

The Environmax planting strategy implemented since the early 1970s has been quite successful in controlling phytophthora diseases (Lim 1980; Ho et al. 1984; Ismail and Mohd 1984). This involves the avoidance of planting susceptible clones in areas conducive to disease development. Under this program, tolerant clones are recommended for planting in pre-demarcated areas. To sustain growth and productivity of susceptible clones already planted, short-term remedial measures using chemicals are recommended. This includes disease forecasting, which has been used successfully to control leaf fall (Lim 1980).

Table 4.1.2. Area planted to rubber in Malaysia, 1995–2000.

Year	Area ('000 ha)
1995	1.688
1996	1.644
1997	1.616
1998	1.556
1999	1.465
2000	1.431

Source: Anon. (2002)

Cocoa

A native of South America who attempted to grow cocoa commercially in Malaysia in the early 1900s did not succeed. Following a report on cocoa by Cheesman in 1948 (unpublished), the Malaysian Department of Agriculture in the late 1940s devoted a great deal of attention to cocoa as a possible crop for diversification (McIntosh 1948). Experimental planting of cocoa using local Trinitario and imported Amelonado failed because of dieback

problems (Haddon 1960). In the 1960s, trial plantings with Upper Amazon as an inter-crop in Peninsular Malaysia and as a mono crop in Sabah proved successful, paving the way for a rapid expansion of cocoa in the country.

Production of cocoa in Malaysia has steadily declined from 9% of world cocoa bean output in 1990–1991 to 2% in 1999–2000. The decline is attributed to the falling price of cocoa, which resulted in growers moving to other crops, especially oil palm.

Table 4.1.3 shows the rapid decline in area under cocoa over the last 10 years. In 1992, the area under cocoa was estimated at 380,000 ha, but by 2001 this had dropped to 70,000 ha, a decline of about 80%.

The dominant *Phytophthora* species on cocoa is *P. palmivora* (A2 mating type) with occasional A1 mating type reported in Sabah (C.L. Bong, pers. comm.). The fungus is present in all cocoa-growing areas. The sporangia are typically caducous, with a rounded base, short pedicel and having a prominent papilla. Length–breadth (L/B) ratio varies from 1.0 to 2.1, but most sporangia lie in the range of 1.4 to 1.6. Some cultures in our laboratory resembled *P. nicotianae*, with ovoid sporangia and L/B ratios of 1.1–1.3 and with no pedicel. *P. heveae* had been isolated from cocoa rhizosphere and was pathogenic to cocoa pods (Lee 1972). In addition, *P. meadii*, *P. heveae* and an unidentified *Phytophthora* species were occasionally isolated from infected pods in Sabah (Liu 1977).

In laboratory tests, several *Phytophthora* species from other host plants were pathogenic to cocoa: *P. cinnamomi* from clove (Lee 1974), *P. capsici* and *P. nicotianae* from capsicum and brinjal (Lee 1972), and *P. botryosa* from rubber (Chee and Hashim 1971). The potential threat of these species to cocoa is significant.

Table 4.1.3 Area planted to cocoa in Malaysia, 1992–2001.

Year	Estate plantings (ha)	Smallholder plantings (ha)	Total (ha)
1992	168,058 (44%)	210,482 (56%)	378,540
1993	145,646 (49%)	154,349 (51%)	299,995
1994	130,232 (48%)	141,107 (52%)	271,339
1995	96,053 (51%)	49,074 (49%)	190,127
1996	73,503 (44%)	94,716 (56%)	168,219
1997	50,270 (36%)	90,629 (64%)	140,899
1998	37,045 (31%)	80,634 (69%)	117,679
1999	27,937 (28%)	72,866 (72%)	100,803
2000	22,439 (30%)	53,327 (70%)	75,766
2001	20,526 (30%)	48,922 (70%)	69,448

Source: Malaysian Cocoa Board

In Malaysia, black pod is the most common phytophthora disease on cocoa. Chan and Lee (1973) reported low incidence of black pod in the early 1970s. The situation was similar in Sabah, where low incidence was attributed to environmental conditions unfavourable for disease development at the time (Liu 1977; Liu and Liew 1975). Incidence and severity of black pod has since increased, due to the planting of highly susceptible clonal materials. In areas of high rainfall and poor agronomic practices, incidence as high as 30% was common (Tey 1983). *P. palmivora* infects pods of all ages, including young cherelles. Lee and Chan (1980) reported that, in localities of high rainfall and poor management, incidence of cherule wilt caused by *P. palmivora* could be as high as 30%. Epidemiological studies of black pod were undertaken by Tey et al. (1986). They showed that incidence of black pod was related to weather conditions and fruiting patterns. Heavy infection occurred during the months of high rainfall, which coincided with the main fruiting season. The abundance of susceptible host tissue under conditions favourable for disease development resulted in high incidence of the disease.

First reported by Chee and Phillips (1971), stem canker is the next most important phytophthora disease on cocoa. Infection starts from anywhere along the trunk, branches or jorquettes. Lesions can also form just above the soil line, and often extend into the soil as well. Incidence and severity of stem canker are closely related to rainfall and management practices. In general, areas with high incidence of black pod also have high incidence of stem canker. An outbreak of stem canker in the mid 1980s in Perlis in northern Peninsular Malaysia was attributed to improper use of drip irrigation. The damp and waterlogged conditions created by the drip around the base of the trees induced the disease to develop (Tey and Musa 1987).

Seedling blight caused by *P. palmivora* was first reported by Chee (1969a). Seedlings of up to 4 months old in polybags could be affected (Lim 1980). Although localised, losses of up to 20% are common (Chan and Lim 1987).

In areas where the incidence of black pod is low, control is achieved by regular removal of infected pods, which are then either buried or burnt. Maintenance pruning is practised to improve ventilation, quicken the drying of pods and stem surfaces, and to prevent disease build-up.

Fungicides are used in most plantations. They include copper-based products such as copper

hydroxide, copper oxychloride, cuprous oxide, copper-mancozeb mixtures, triphenyltin acetate, etridiazole, metalaxyl, and fosetyl aluminium. Depending on the size of trees, most plantations used either pressurised knapsack sprayers or motorised mist blowers. Excellent control of black pod was achieved by injecting the trunk of affected trees with neutralised phosphorous acid (Tey and Lee 1994). Continued exposure of the pathogen to sublethal doses of systemic fungicides can lead to the development of resistant strains. This was demonstrated by Tey (1984) when he exposed mycelium to sublethal doses of metalaxyl and milfuram.

Considerable progress has been made to develop high-yielding varieties with favourable secondary characters such as disease tolerance (Chong and Shepherd 1986; Tey 1987; Tiong and Kueh 1986). Current research includes clonal selection for disease tolerance and biological control studies.

Durian

Southeast Asia is the centre of origin of *Durio* species, with the majority originating from the island of Borneo. There are some 28 *Durio* species in Malaysia, of which about 11 are edible. *Durio zibethinus* is the only species cultivated commercially. All the registered 'D' clones are from this species. There are still many wild and semi-wild varieties of *D. zibethinus* waiting to be assessed in proper trials on performance, susceptibility and yield. Area under durian has steadily increased since the early 1990s (Table 4.1.4). The drop in farm prices of first grade durian in the last three years has put a damper on durian production. Many farms are being neglected, resulting in the increased incidence of pests and diseases including phytophthora diseases.

Table 4.1.4 Area under durian cultivation in Malaysia, 1990–1997.

Year	Area (ha)
1990	57,000
1991	62,000
1992	62,000
1993	83,000
1994	107,000
1995	108,000
1996	110,000
1997	112,000

Source: Department of Agriculture, Malaysia

The dominant species attacking durian is *Phytophthora palmivora*, although more than one

species may be involved. L/B ratio of the sporangia varies from 1.3 to 2.2, with majority falling between 1.8 and 2.1.

All parts of the durian tree are attacked by *P. palmivora*: the trunk, twigs, branches, fruit, leaves, flowers and the underground portion of the stem and roots. Entry of pathogen is through wounds caused by mechanical injury or through natural openings (Lee 1999). With regular inspection, above-ground lesions can be easily treated. Treatment is difficult when the lesions have penetrated deep into the wood or have completely girdled the tree. Stem and root lesions formed below ground are difficult to detect or treat. Fruit rot is an important disease and, depending on weather conditions, 20–30% of the fruits in an orchard may be affected.

The incidence of durian canker is high in most orchards. In a survey of six locations in Penang, 30% of nearly 2000 trees examined were severely affected by stem canker (Hashim et al. 1991). This figure is representative of most of the orchards in Malaysia. If one assumes that 10–20% of mature durian trees in the country are affected with canker and 50,000 hectares are of fruit-bearing age, there will be a total of half to one million infected trees in the country. Untreated trees will eventually die.

Control of the disease is limited to foliar application of fungicides in the nursery to protect the young seedlings (Chan and Lim 1987) and bud-wood nurseries, and curative treatment to control stem canker in the field. This is achieved through extensive and laborious tree surgery to remove the infected bark and the underlying wood tissue, followed by painting with protective and curative fungicides such as fixed copper fungicides, dimethomorph, triphenyltinacetate, oxadixyl, metalaxyl and fosetyl aluminium. Lim and Yassin (1985) found metalaxyl and fosetyl aluminium to be readily translocated to nearby tissues when these chemicals were painted onto the surface of the scraped branches. Lee et al. (1988) reported excellent control of phytophthora in durian seedlings when the seedlings were trunk injected with phosphorous acid. Trunk injection of mature trees with metalaxyl and fosetyl aluminium (Lee 1994) and phosphorous acid (Lim and Lee 1990) also provided good control. Foliar application with 0.4% phosphorous acid also gave excellent protection of one-year-old seedlings (Table 4.1.5).

While all *D. zibethinus* clones are susceptible, there is variability in susceptibility. Screening of clonal materials through wound inoculation showed that D24 and D66 were the most susceptible while D2 and D10 were the least susceptible (Tai 1973). Nik

(2000) also reported mixed reactions of durian clones to phytophthora. In an attempt to overcome the disease, several hybrid clones have been developed by the Malaysian Agricultural Research and Development Institute (MARDI) in recent years, some with very promising anti-phytophthora properties. In a study of 10-year-old clonal hybrids subjected to heavy inoculum pressure and high annual rainfall, Lee (1999) found MDUR 79, MDUR 88 and MDUR 78 to be the least susceptible. These were hybrids derived from D10 and D24 crosses. In the same study, the most susceptible clone was D24.

Table 4.1.5 Effect of phosphorous acid on control of durian stem canker.

Treatment	Lesion length (mm) ^a
Foliar spray	16.6
Soil drench	44.5
Control	71.9

^a Mean of eight one-year-old seedlings

D24 is a tree that grows vigorously and has excellent fruit quality. The extensive planting of this clone, with its vigorous growth, thick foliage and high branching system, has contributed significantly to the incidence and severity of the disease throughout the country in recent years. However, when bark of D24 seedlings was artificially inoculated without wounding, the stem remained healthy. This indicated that mechanical injury was an important factor in disease initiation. This observation has led Nik and Lee (2000) to develop a rain-fast wound dressing specifically for durians. Wounds treated with this dressing were protected against infection in the field. The protection could last for at least six months, long enough for the wounds to be naturally healed.

In an extensive study on the potential of tolerant rootstock to overcome patch canker in durian, Lee (1999) studied the possibility of using *Durio lowianus* as rootstock. Excellent survival of D24 trees grafted onto *D. lowianus* rootstock in a naturally infested field after 13 years of planting indicated that *D. lowianus* has good potential for commercial use to prevent premature death due to *P. palmivora*. Nearly 50% of D24 trees grafted onto normal rootstock died of canker within 13 years while close to 100% survived when they were grafted onto *D. lowianus* rootstock.

The use of suppressive soil for controlling phytophthora diseases has been well documented (e.g. Broadbent and Baker 1974; Ko and Nishijima 1985; Ko and Shiroma 1989). Lee (1999) reported possible suppression when durian trees were planted in limestone soil high in soil pH, cation

exchange capacity, exchangeable calcium and micronutrients such as Mn, Zn and Cu. The presence of relatively high copper content in these soils is interesting because copper ions are strongly fungitoxic to *P. palmivora*. While copper deficiency causes dieback of durian trees.

Pepper

Piper nigrum L is native to the state of Kerala in India. Hindu migrants to Indonesia first introduced the crop into Southeast Asia as early as 100 BC. India and Indonesia are the main producers of pepper, accounting for more than 50% of world production. In recent years, Vietnam has become an important producer as well.

In the early 1800s, the crop spread to Sarawak, which is now the main pepper-producing state in Malaysia (Table 4.1.6). Pepper orchards are generally small, averaging about 0.25 ha or 400 vines, and situated on hill slopes, often without ground cover. The high-yielding but susceptible Kuching variety is the most widely cultivated variety in Malaysia. Average yield is between 2 and 3 kg of dried pepper per vine, with some progressive farmers reporting a yield of 4 kg or more (Anon. 2002).

Pepper requires a tropical climate with well-distributed annual rainfall of 2000–4000 mm, a mean air temperature of 25–30°C and relative humidity of 65–95%. It grows best at altitudes below 500 metres, but may grow up to 1500 metres above sea level, and on soils ranging from heavy clay to light sandy clay. Soils should be deep, well drained and with good water-holding capacity to deal with water stress during the dry period.

Phytophthora capsici affects the leaves, spikes, berries, branches, climbing stems, underground stems and roots, i.e. all parts of the pepper vine. Initiation of infection takes place during wet weather when black necrotic spots with typical fimbriate margins develop on the lower leaves as a result of rain splash. These infected leaves subsequently drop off, resulting in the built up of soil inoculum. Roots and underground stem infection is indicated when the leaves turn pale and flaccid. Leaf and spike fall indicate a late stage of infection. Eventually, the vine is completely defoliated and is left standing with only the climbing stems and lateral branches. Infection may start at soil level or at any point along the underground stem to a depth of 20 cm. Lesions on stem and roots are dark brown in colour with a sharp margin of demarcation.

Phytophthora capsici grows best in a humid environment of 25–30°C and a pH of 5.5 to 6.0. The

identification and taxonomy of this species has been well described by Alizadeh (1983) and Alizadeh and Tsao (1985). In the 1970s, when pepper was widely grown in Johor, the species frequently isolated was *P. nicotianae*. Tsao (1986) also reported the presence of *P. nicotianae* in Thailand. From his study of pepper phytophthoras from around the world, Tsao (1986) concluded that there was no typical *P. palmivora* on pepper.

The biology, spread and control of pepper foot rot in Sarawak had been studied by Kueh (1977) and Kueh and Khew (1982). Inoculum is spread by rain splash, root contact, snails (*Achatina fulica* and *Hemiplecta crossei*), and wooden posts from infected fields, farm tools and man. The fungus could survive in soil in the absence of a host for at least 18 months. Fungal propagules were found mainly in the first 15 cm of the soil profile, with very low counts at a depth of 30–45 cm. The optimum soil moisture for survival was 25–45% water-holding capacity and soil pH 6.5–7.0.

Table 4.1.6 Area (ha) under pepper cultivation in Malaysia, 1999 and 2000.

State	1999	2000
Sarawak	12,196	12,996
Johor	43	43
Sabah	48	45
Total	12,287	13,084

Source: Department of Statistics, Malaysia

Lee (1973) studied the mating types of pepper isolates from Johor and Sarawak, and concluded that the Johor isolate (probably *P. nicotianae*) was of the A1 mating type while the Sarawak isolate was of the A2 mating type. In addition, an atypical strain from Sarawak that formed oospores in single culture was reported by Turner (1962).

Lee (1973) reported the use of culture filtrate as a possible method to screen for resistance. From his study, two distinct groups of *Piper* spp. could be differentiated: the resistant group consisting of *Piper colubrinum* and *Piper sarmentosum*, and the susceptible group consisting of *Piper nigrum* varieties Kuching, Bangka, Djambi, Belantung and Uthirancotta, with the Kuching variety being the most susceptible and Uthirancotta the least susceptible. Similar results were obtained by Kueh and Khew (1980) when they used different fungal propagules as inoculum. Attempts to use *P. colubrinum* as resistant rootstock had met with little success due to late incompatibility and high susceptibility of *P. colubrinum* to other root diseases. Development of resistant planting material is

urgently needed. Certain varieties showed some tolerance, but infection and spread of disease in the field was only retarded rather than controlled. The ideal strategy for foot rot control is to adopt an integrated approach that involves cultural practices, chemical and biological control, and the exploitation of host resistance (Kueh and Khew 1980)

Future Directions

Public research institutions funded by the federal government have been established to carry out research on specific crops in Malaysia. For example, research on rubber is carried out by the Malaysian Rubber Board, oil palm by the Malaysian Palm Oil Board and cocoa by the Malaysian Cocoa Board. Research on all other crops is carried out by MARDI and universities involved in biological sciences. Research on forestry is carried out by the Forest Research Institute of Malaysia. The departments of agriculture in Sabah and Sarawak have their own research centres to cater for their own regional needs. In these institutions, priority has always been given to phytophthora research. Directions that need to be developed or further strengthened are:

1. accurate detection and identification of species and strains within species through DNA based diagnostics and DNA fingerprinting
2. studies on the nature and diversity of *P. palmivora* and to develop, if feasible, national breeding and selection programs for crops such as rubber, cocoa, durian and pepper
3. integrated control of phytophthora diseases, including the use of resistant genes from wild plant species
4. training of plant pathologists in specific area of phytophthora research (isolation, identification, ecology, biological control, epidemiology, and disease management)
5. regional collaboration through the formation of a phytophthora working group for Southeast Asian countries sharing common phytophthora problems.

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4.2 Phytophthora Diseases in Indonesia

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Abstract

This review summarises the species of *Phytophthora* recorded in Indonesia, their hosts, distribution, and current control measures. Some advances in research and control of phytophthora diseases have been made, but there is still a long way to go before sustainable disease-management practices are available for the wide range of diseases caused by different species of *Phytophthora*.

Introduction

Indonesia is often referred to as the world's largest archipelago, consisting of 17,000 islands (6000 inhabited) scattered around the equator. It has a tropical, hot, humid climate with more moderate conditions prevailing in the highlands. Terrain is mostly coastal lowland, whereas the larger islands have interior mountains. Having a tropical climate with high levels of rainfall and humidity in most areas, several phytophthora diseases cause significant damage and are difficult to control.

Phytophthora spp. cause important diseases in agricultural, horticultural and industrial crops in Indonesia. At least 11 species of *Phytophthora* are reported to cause economic losses in Indonesia. *Phytophthora palmivora* has been identified as the most economically important *Phytophthora* species in Indonesia. It causes diseases on the largest number of economically important plant species (Table 4.2.1). In fact, it has been recorded as attacking more than 138 plant species. *Phytophthora palmivora* causes approximately 25–50% yield loss on cocoa, whereas *P. capsici* causes 52% yield reduction in pepper. However, the disease losses on most plants have not been accurately quantified.

Phytophthora spp. infect various parts of plants including roots, stems, leaves, and fruits. Disease symptoms vary depending on the host, species involved and the prevailing conditions.

Phytophthora cinnamomi is known to infect stems causing bark canker in cinchona and cinnamon, whereas *P. palvimora* infects all parts of cocoa, causing root rot, stem canker, pod rot, leaf blight and chupon blight.

Also known as a water mould, the life cycle of *Phytophthora* reflects adaptation to an aquatic environment. A tropical climate with prolonged wet conditions and relatively stable temperatures is very conducive for the pathogen. Disease epidemics normally occur during the wet season. Sporangia can either germinate directly by forming a germtube, or differentiate into up to 50 biflagellate zoospores. Using their flagella, the zoospores can move actively in water for short distances before they encyst and germinate to initiate infections. In the soil, zoospores are attracted to the roots of plants. This mobility of zoospores to their host is a very important characteristic for the local spread and development of epidemics by *Phytophthora* species.

This review summarises the species of *Phytophthora* recorded, their hosts, distribution, and current control measures in Indonesia. The biology, epidemiology and control of the two most important species, namely *P. palmivora* and *P. capsici*, will also be presented. The review concludes with a discussion on the future research and implementation of integrated management to control phytophthora diseases.

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Table 4.2.1 *Phytophthora* species, economically important hosts and diseases caused in Indonesia

<i>Phytophthora</i> species	Host	Scientific name	Diseases caused	Regions where pathogen has been recorded	Reference
<i>P. cactorum</i>	Apple Avocado	<i>Malus pumila</i> <i>Persia americana</i>	Collar rot Root rot, stem canker	Java	Sukirman and Semangun (1972) Triharso et al. (1975)
<i>P. capsici</i>	Chili Pepper	<i>Capsicum</i> spp. <i>Piper nigrum</i>	Fruit rot Foot rot	Java, Sumatra, Bangka, Kalimantan	Muller (1936) Anon. (1987)
<i>P. cinnamomi</i>	Cinchona Cinnamomum	<i>Chincona ledgeriana</i> <i>Cinnamomum burmannii</i>	Stem canker Stem canker	Java, Sumatra	Djafaruddin (1975) Winarto et al. (1980)
<i>P. citricola</i>	Cinchona	<i>Chincona ledgeriana</i>	Seedling dieback	Java	Semangun et al. (1977)
<i>P. citrophthora</i>	Citrus	<i>Citrus</i> spp.	Foot rot, gummosis	Java, Sumatra, Kalimantan	Toxopeus (1932) Muller (1939) Anon. (1987)
<i>P. colocasiae</i>	Taro	<i>Colocasia esculenta</i>	Leaf blight	Java	Semangun (1991a)
<i>P. infestans</i>	Potato Tomato	<i>Solanum tuberosum</i> <i>Lycopersicon esculentum</i>	Late blight Leaf blight, fruit rot	Java, Sumatra, Bali, Lombok, Sulawesi	Thung (1947), Suhardi et al. (1976) Anon. (1987)
<i>P. nicotianae</i>	Tobacco	<i>Nicotiana tabacum</i>	Black shank	Java, Sumatra	Thung (1938) Hartana and Soepeno (1978)
	Citrus Pineapple Roselle Vanilla	<i>Citrus</i> spp. <i>Ananas comosus</i> <i>Hibiscus sabdariffa</i> <i>Vanilla planifolia</i>	Foot rot Heart rot, root rot Black foot rot Pod rot	Java, Sumatra, Bali	Semangun (1991b) Toxopeus (1934) Oka and Prajati (1972) Anon. (1987)
<i>P. palmivora</i>	Cacao Citrus Coconut	<i>Theobroma cacao</i> <i>Citrus</i> spp. <i>Cocos nucifera</i>	Pod rot, stem canker Gummosis Bud rot, premature nutfall	All regions	Purwantara (1987) Triharso et al. (1975) Wahyuni and Hadisutrino (1976) Purwantara and Darmono (2003)
	Durian Orchid Papaya Rubber	<i>Durio zibethinus</i> <i>Vanda</i> spp. <i>Carica papaya</i> <i>Hevea brasiliensis</i>	Patch canker Black rot Root rot, fruit rot Black stripe, leaf fall		Prayudi (1988) Dwiastuti (1985)
<i>P. porri</i>	Shallot	<i>Allium ascalonicum</i>	Blight	Java	Anon. (1987)

Diseases of Major Economic Importance

Phytophthora diseases in cocoa

Cocoa is an important commodity for Indonesia. The total area planted to cocoa was 532,000 ha in late 1999. Just over 70% of cocoa farmers are smallholders. Indonesia is the world's third largest cocoa exporter. It produces 335,000 tonnes/year, which is valued at USD294 million. *Phytophthora palmivora* is a serious pathogen of cocoa, causing pod rot, stem and cushion cankers, leaf, chupon and seedling blights and sudden death (Sri-Sukanto 1985; Purwantara 1987). At the beginning of the last century, canker was very serious in Java, leading to the eradication of the very susceptible Criollo types of cocoa (Van Hall 1912, 1914). However, canker is no longer a menace in this area since Criollo has been replaced by Forastero types (Tollenaar 1958). In most areas, direct losses by pod infection leading to black pod rot are the most common cause of the problem. Newly set fruit up to fully mature pods are susceptible to infection.

There is a positive correlation between disease intensity and the number of pods per tree. Higher-yielding trees had a higher percentage of black pods than lower-yielding ones, as most of the latter escaped infection (Tollenaar 1958). When the incidence is high, an abundance of sporangia is produced. This, in turn, makes it more difficult to control the disease than when the incidence of pod infection is low. For the same reason, disease control becomes gradually easier in an area where the control measures have been executed systematically year after year.

Production of spores and the risk of infection are increased by high humidity. The incidence of phytophthora diseases can be very high in wet years and in humid areas. A combination of high rainfall and high humidity during the crop season will lead to severe losses. In West Java, cocoa plantations in areas with an annual rainfall of approximately 4000 mm at 400–600 m above sea level suffered very high incidence of pod rots and cankers (Purwantara 1990). Even in the absence of rain, infections still occur in these areas, as the humidities of nearly 100% that occur for a few hours during the night provide enough free water to initiate infection (Purwantara and Pawirosoemardjo 1990; Purwantara 2003). Poor drainage of plantations, high humidity due to heavy canopies, and low branching of trees increased disease incidence in mountainous areas of Java. Pruning of cocoa and removal of low branches provide some reduction in disease incidence. For

this reason, pod rot usually becomes increasingly serious as soon as the canopy has closed, this occurring after 5 to 7 years (Van Hall 1912).

Originally, disease control was attempted by removing the newly infected pods and burying them. Removal should be done every other day, as new spores are produced on pods within 2 days after the first symptoms are visible (Tollenaar 1958). However, even daily removal of the infected pods did not reduce disease incidence below economic threshold levels (A. Purwantara, unpublished data). It seems that infected pods are not the only source of infection in plantations. Other sources, including infected cushions and cankers, soils and insects are part of the disease cycle (Konam 1999).

Chemical spraying using copper-based fungicides has been practised in several cocoa plantations. However, because of wash-off in the wet season, these sprays provide only limited protection. Trunk injection with phosphonates provides good control of pod rot and stem canker in East Java (Y.D. Junianto, unpublished data), but this control technique is not widely adopted by growers. They are reluctant to drill holes in the trees because of they have limited information about the healing process of wounds from multiple and regular injections. The current recommendation for controlling the diseases is integrated management, including the reduction of inoculum from the soil by ground-cover management and removal of tent-building ants, adoption of wide plant spacing and regular pruning to reduce humidity in the canopy, removal of infected pods, frequent harvest to remove sources of secondary inoculum from the canopy, and trunk injection with phosphonate.

Phytophthora diseases in coconut

The production of coconut and copra are extremely important activities in Indonesia. Annual copra production is 2.342 million tonnes (26% of world production) from 1.384 million ha (32% of world coconut area). The bulk of production is by smallholders, with other cash and food crops generally planted under coconut, which notably serves as a shade tree for cocoa. Breeding for improved varieties represents a national priority. The improved hybrid variety PB121 (MAWA) was successfully adopted by many smallholders, but in the early 1980s a disease of coconut causing budrot and premature nut fall was identified on this variety and now rates as the most significant disease affecting coconut production in the country.

Rots caused by *Phytophthora* species lead to palm death (by bud rot) and/or yield reduction (by

premature nut fall) (Waller and Holderness 1997), and are the major disease problems affecting coconut in Indonesia (Lolong et al. 1998). While most of the coconut-growing regions of the world are affected by phytophthora rots, Indonesia and the Philippines are the worst affected (Renard 1992). In Indonesia and the Philippines, *P. palmivora* seems to be the main causal agent of disease (Blaha et al. 1994). Coconut bud rot has an irregular distribution in the field, but the highest incidence seems to correlate with the wettest areas (Waller and Holderness 1997) and with plantings of the susceptible hybrid, PB121 (MAWA).

Bud rot and nut fall were first reported in Indonesia in 1985, the causal agents being identified as *P. palmivora* and *P. nicotianae* (Bennett et al. 1986). During this time, outbreaks of the disease resulted in severe damage to plantations (Renard 1992). Since that time, almost all areas planted to coconut in Indonesia have suffered serious damage from bud rot, with losses above 80% (Darwis 1992). The severity of disease is linked to the introduction of high-yielding hybrid breeding lines from West Africa (MAWA). These are highly susceptible to phytophthora (see also Chapter 6.2). In Indonesia, although *P. palmivora* seems to be the main causal agent of bud rot and nut fall in coconut (Blaha et al. 1994; Waller and Holderness 1997), *P. arecae* and *P. nicotianae* have also been found in association with these diseases (Thevenin 1994) in a small number of cases. Nut damage is usually most severe in immature bunches during the rainy season. *Phytophthora* spores proliferate and then spread horizontally (by contact between bunches) or vertically (between nuts within a bunch) (Renard and Darwis 1992). The diseases cause extensive losses of both stands and nut production. In some areas, stand losses of 43% can occur due to bud rot. Premature nut fall, which is the more common disease, affects nuts 3–7 months old (Lolong et al. 1998), and can cause losses of 50–75% (Brahmana et al. 1992). The incidence of bud rot is higher in the lowland areas of Indonesia than in the highlands. Resistance among coconut varieties to infection and damage by phytophthora varies with location, and therefore it is recommended that several varieties be planted to minimise the risk of damage caused by the pathogen (Mangindaan et al. 1992).

From field observations and inoculation studies, some varieties have been found to be resistant in Sulawesi, but knowledge of the variation in the pathogen populations is required for successful resistance breeding programs. The susceptibility of PB121 to *P. palmivora* has been linked to its parental lines, yet these parental lines continue to be used in

breeding programs due to their favourable early and high-yielding characteristics. National plant breeding programs are on the way to ensure that the next generation of recommended coconut varieties planted in Indonesia is not susceptible to *P. palmivora*. An increased understanding of this disease will enable the development of improved disease-management procedures

Foot rot in pepper

Phytophthora capsici Leonian causes the most destructive and economically significant disease of black pepper (*Piper nigrum* L.). The fungus attacks all parts and growth stages of the black pepper plant. If it attacks the root or collar, it causes sudden death. This disease was first reported in Lampung in 1885, and has been known as foot rot disease since 1928 (Muller 1936). The causal agent was first identified as *P. palmivora* var. *piperis* (Muller 1936), then in 1985 it was recognised as *P. palmivora* MF4 (Tsao et al. 1985) and later renamed *P. capsici sensu lato* (Tsao and Alizadeh 1988). Nowadays, the disease is found in almost all areas where pepper is cultivated in Indonesia.

Pepper (black and white final products) is the seventh most important export income earner for Indonesia. The total area planted is about 136,450 ha. The crop is produced by over 132,000 farmers, most of whom are smallholders. They care for and control their cultivations when the pepper price is high, but neglect them if the price falls. All cultivated pepper varieties grown in Indonesia are susceptible to the disease. Vines more than 3 years old seem to be the most susceptible to foot rot (Holliday and Mowat 1963).

Population Biology

Some understanding of the biology and epidemiology of phytophthora diseases has been achieved. Populations of heterothallic *P. palmivora* attacking coconut and cocoa in Indonesia consist of only one mating type (A1). In contrast, populations from papaya consist of mating type A2. No oospores have been reported in the field so far. Molecular analysis of this *P. palmivora* population showed limited genetic diversity amongst isolates originating from coconut. *P. palmivora* affecting cocoa was shown to be genetically distinct from that isolated from coconut and this distinction was confirmed by pathogenicity assessments. Two mating types have been reported in *P. capsici* attacking black pepper (Manohara et al. 2002). However, the importance of sexual reproduction in enhancing genotypic diversity in *P. capsici* populations and the importance of the formation of

oospores as long-term survival structures have not been determined

Control of *Phytophthora* Diseases in Indonesia

Most phytophthora diseases can be cost-effectively controlled only through a well thought out integrated disease management program that incorporates, in an appropriate way, the several control measures that are available. There is still considerable scope for research into several aspects of the resistance of plants and the genetics of the pathogen, especially in understanding mechanism of pathogenesis, host specificity of the phytophthora pathogens and in the development of sustainable and cost-effective integrated disease-management practices.

The impact of phytophthora disease can be reduced through manipulation of the environment, such as by reducing humidity in orchards through pruning, weeding and good drainage. Sanitation and crop rotation also provide good control, such as in black shank of tobacco, where crop rotation and monitoring of the pathogen population in the soil through baiting with tobacco leaves has been implemented since before the 1940s (Semangun 1991a,b). Planting resistant varieties will be the best option for controlling the disease. However, such material is not available for all disease systems and against all species of *Phytophthora*. Selection and breeding for resistance to phytophthora have provided an effective means of controlling some phytophthora diseases of economic importance in Indonesia. In some plant species, such as in tobacco, resistance has been identified. Nevertheless, the genetics of resistance in many tropical crops, especially tree crops, is not fully understood and needs significantly more investigation. In disease systems, such as black pepper-*P. capsici*, sources of resistance appear to be limited. Introductions of plant materials with higher levels of resistance to phytophthora diseases are urgently needed. Late blight of potato and black shank of tobacco have been sufficiently controlled by moderately resistant cultivars. Chemical control relies on copper-based fungicide as protective measures, and systemic fungicides such as metalaxyl and phosphonates.

Biological control may become an alternative control for phytophthora diseases, as it is considered as an environmentally safe form of disease control. In an attempt to reduce the use of fungicides in response to increases in price and environmental concerns, research on biological control has been conducted

for several *Phytophthora* species. Various fungi, actinomycetes and bacteria have been isolated and proven to control the pathogen in glasshouse trials. However, the effectiveness of these biological control agents needs to be demonstrated and validated in the field.

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4.3 Phytophthora Diseases in Thailand

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Abstract

Phytophthora diseases have been recorded on durian, rubber, black pepper, cocoa, citrus, potato and pineapple in Thailand. *Phytophthora palmivora* is the predominant species and is found on many different crops. It has a wide host range and shows considerable morphological variability. Epidemiological studies indicate that rainfall has a significant influence on disease development. Control of phytophthora diseases is difficult, and research efforts are directed towards using biological and chemical control as part of integrated disease control practices.

Introduction

Many *Phytophthora* species have been reported in Thailand: *Phytophthora nicotianae*, causing root and fruit rot of citrus (Wichiencharoen 1990); *P. palmivora*, causing pod rot of cocoa (Kasaempong 1991) and patch canker and fruit rot of durian (Bhavakul and Changsri 1969); and other crops as shown in Table 4.3.1.

Root and Stem Rot of Durian

Durian has been grown commercially in Thailand since 1800, and there are at least 68 cultivars grown in the area around Bangkok and Nonthaburi. In 1942, there was a serious flood in the area that damaged most of the durian plantations. Since then, farmers recultivated durian by propagating the E-Luang cultivar as a monocrop. In 1966, root and stem rot of durian was reported on 20-year-old trees and, in 1967, durians in Chantaburi Province

showed symptoms of root rot, especially when grown near irrigation lines and canals.

The causal agent was identified as *P. palmivora* (Chee 1969). Monthong, E-loung and Chanee cultivars were reported as being susceptible to *P. palmivora*. The symptoms that appeared on roots and stems can be described as dark brown to black discoloration with rotting root and bark on the base of the trunk. In years when there is a long wet season and turbulent strong winds, the symptoms can be found on twigs as high as 10 m above the ground. Mycelium can infect leaves and young shoots and produce white, fluffy mycelium on the lesion under humid conditions. Some investigations have reported that beetles, termites and ants may be involved in carrying the fungus up into the canopy of the tree.

Root and Stem Rot of Black Pepper

The causal agents have been reported as *P. palmivora*, *P. nicotianae* and *P. capsici*. The pathogens infect the roots of black pepper vine below the soil line. The first symptoms appear as dark brown to black lesions at the tip of the young root. Nodes on the upper part can be removed easily. Small lesions enlarge and merge into larger lesions and turn black with age. Infected leaves, pedicels and flowers show rot symptoms, while the fruit turns brown, dries and wilts. If young vines are infected, the plants die in 1–2 months. If the pathogen infects older vines, the plants show a significant decline in yield before dying.

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Table 4.3.1 *Phytophthora* species reported from different plants in Thailand.

<i>Phytophthora</i> species	Host	Common name	References
<i>P. botryosa</i>	<i>Hevea brasiliensis</i>	rubber	Suzui et al. (1979)
<i>P. capsici</i>	<i>Piper nigrum</i>	black pepper	Tsao and Tummakate (1977)
<i>P. infestans</i>	<i>Solanum tuberosum</i>	potato	Tucker (1933)
<i>P. meadii</i>	<i>Hevea brasiliensis</i>	rubber	Chee and Greenwood (1968)
<i>P. nicotianae</i>	<i>Citrus</i> spp. <i>Durio zibethinus</i> <i>Ananas comosus</i> <i>Piper nigrum</i>	citrus durian pineapple black pepper	Suzui et al. (1979) Suzui et al. (1979) Suzui et al. (1979) Silayoi et al. (1983)
<i>P. palmivora</i>	<i>Piper nigrum</i> <i>Durio lowianus</i> <i>Durio zibethinus</i> <i>Hevea brasiliensis</i> <i>Theobroma cacao</i> <i>Euphorbia longana</i> <i>Mangifera indica</i> <i>Ananas comosus</i>	black pepper wild durian durian rubber cocoa longan mango pineapple	Krengpiem et al. (1989); Kunloun (1967); Tsao and Tummakate (1977) Kumjaipai (1974) Suzui et al. (1979) Tsao et al. (1976) Chomenansilpe et al. (1983) Bhavakul et al. 1997 Kueprakone et al. (1986) Suzui et al. 1979)

Coconut Nut Drop

Malayan yellow dwarf, an imported coconut cultivar, showed heavy nut fall in a breeding plot at Chumporn Horticulture Research Centre in 1968. Investigations revealed that *P. palmivora* had infected the coconut. Symptoms on the nut were found at the base of the pedicel attached to the outer carp. The pathogen can infect the fruit at 2–8 months stage, and often the disease lesion starts from the pedicel base down inside the fruit to the young shell. In moist conditions, fluffy white mycelium can be seen at the early stages of infection but not in the later stages. Infected nuts die prematurely. Symptoms can also be found on shoots of the seedling while it germinates. Studies on the host range revealed that mangosteen, tangerine, lime, coffee, rambutan, black pepper, cocoa and pineapple can be infected with *P. palmivora*. So far, nut drop has not been reported from any other coconut cultivars in Thailand.

Black Rot Disease of Vanilla

Vanilla is a crop of economic value due to its aromatic flavour that is used in the manufacture of chocolate, ice-cream, soft drinks, cakes and snacks. Black rot disease of vanilla is the most severe disease limiting vanilla production. The disease was first found at Maehae Highland Agriculture Station in Chiangmai Province. Symptoms first appeared as yellowing on leaves and stems. The pathogen, *P. palmivora*, infects the roots and foot, developing into black rot in the roots, foot, stem and leaves. Furthermore, the causal agent can directly infect the

shoot and leaves, again leading to black rot and death of the plant. The disease can become epidemic during prolonged periods of high humidity in the rainy season. Rain splash helps the dispersion of zoospores from infected soil up to the plant.

Longkong (longan) root rot

The symptoms of longkong root rot appeared on 2-year-old longkong seedlings on langsat root stock at Chantaburi Province in 1999. Characterisation of the sporangium, chlamydospores and mating types revealed that the pathogen was *P. nicotianae*.

Leaf Fall and Black Stripe in Rubber

Rubber is an important crop to Thailand, especially in the south, where average annual rainfall is 2000–3000 mm and average temperature is 28±2°C. There are several diseases caused by different *Phytophthora* species that limit rubber production in Thailand. The major diseases are phytophthora leaf fall and black stripe.

Leaf fall, caused in Thailand by *P. palmivora*, is characterised by individual leaves turning yellow. The lesions turn dark brown to black and often show white spots of coagulated latex in the centre of the lesion. Leaf fall is most common soon after the monsoon season has started and may give rise to serious defoliation. In addition to the leaves, the fruit may also be infected. The fruit may be covered with sporulating mycelium during periods of high humidity. In contrast to the leaves, the infected fruit turn dark but remain attached to the tree.

Black stripe is a disease of the tapping panel caused by *P. palmivora* and *P. botryosa*. Sunken discoloured areas appear on the tapping panel, and when the bark is cut away, characteristic vertical black lines become apparent. The disease develops rapidly in wet weather.

Disease surveys in rubber-growing areas in 1976, including 3 provinces in the east and 14 in the South, indicated that 10% of the total area was infested (Kajornchaiyakol 1977). Phytophthora diseases were prominent in the western coast of the South due to long periods of wet, humid weather with few periods of sunshine (Kajornchaiyakol 1977). However, phytophthora diseases have, in recent years, been less troublesome than previously, due to the introduction of more-resistant clones in the affected areas (Chantarapratin et al. 2001).

Root Rot, Gummosis and Brown Rot in Citrus

Thailand is among the largest producers of a wide range of different citrus fruits in Southeast Asia. However, due to the prevailing wet climatic conditions, phytophthora is a major impediment to production. In Thailand, citrus is grown by smallholders as well as on large plantations. Root rot and foot rot are common in many citrus species, especially after prolonged periods of wet weather. Gummosis, a rotting of the bark due to phytophthora growing into the cambium and producing a necrosis, is often accompanied by the exudation of water soluble gum. Brown rot of the fruit is common under wet conditions.

Table 4.3.2 Major phytophthora diseases in southern Thailand.

Crop	Disease	Disease occurrence ^a (%)
Rubber	Leaf fall and black stripe	10 ^b
Durian	Root and stem rot	3.5
Citrus	Root and stem rot	0.5
Robusta coffee	Root and stem rot	0.4

^a Average percentage of infested area per year (Source: Department of Agricultural Extension, southern unit, Songkhla).

^b Infested area surveyed in 1976 (Kajornchaiyakol 1977)

Occurrence of *Phytophthora*

Although root and stem rot in fruit crops caused by *Phytophthora* was endemic in the south (Table 4.3.2), the infested area was less than 10% of the total

growing area in each year. This was an average from reports on plant pests in southern Thailand during 1998–2001 in the Thailand Department of Agricultural Extension annual report. However, in certain years the damage to durian (Table 4.3.3) was high in particular areas: 51%, 41% and 38% in Chumporn, Ranong and Surat Thani provinces, respectively, in southern Thailand in 2001. Although the percentage infestation by phytophthora disease in citrus was low in southern provinces, in other areas the disease had devastated particular orchards (Figure 4.3.1). In addition, the damage by phytophthora disease in coffee was insignificant because the disease incidences were reported only in 2001 with 0.4% infestation.



Figure 4.3.1 Phytophthora stem rot in a Shogun mandarin orchard in southern Thailand.

Table 4.3.3 Incidence of root and stem rot in durian in southern Thailand, 2001.

Province	Growing area (ha)	Infested area (%)
Chumporn	21,490	51
Ranong	3,732	45
Surat Thani	6,174	34
Nakan Si Thammarat	20,532	27
Phangnga	2,150	38
Krabi	1,400	20
Phuket	592	7

Source: Surat Thani Biological Pest Management Centre, Department of Agricultural Extension.

Considerable amounts of fungicides have been used to manage phytophthora diseases in fruit crops, particularly in durian. Biological control measures using antagonistic fungi (*Trichoderma harzianum*) to suppress phytophthora infestation has increased in the past 3 years. The biological control agents were

distributed to farmers through Surat Thani and Songkla Biological Pest Management Centre, Department of Agricultural Extension. Selection and utilisation of resistant varieties to control phytophthora root rot in durian is continuing. Screening for resistance to *P. palmivora* in selected durian seedlings (Figure 4.3.2) for the selection of more-resistant rootstock has been developed by the Tropical Fruit and Plantation Crop Research Centre, Prince of Songkhla University since 1997. A few promising clones have been detected from Nakorn Sri Tammarat, Songkhla and Narativat provinces (Kanjamaneeesathian et al. 2000).

In Thailand, the mating type of *P. palmivora* is reported as A1, *P. nicotianae* is both A1 and A2, and *P. botryosa* is A1 and A2. *P. palmivora* isolates obtained from cocoa and durian showed variability in their colony characteristics (Kasaempong 1991).

Research on *Phytophthora* in Thailand

Phytophthora diseases are a major constraint to the production of many crops in Thailand. The most common *Phytophthora* species are *P. palmivora*, *P. nicotianae* and *P. botryosa*. *Phytophthora* isolates show a high level of variation and wide host range. Epidemiological studies of *Phytophthora* pathogens conducted in durian, citrus, and cocoa indicated that high levels of rainfall in many parts of Thailand are a major contributing factor to disease incidence.

Thailand has, in the more recent past, built up a considerable level of experience in plant pathology and mycology, including of *Phytophthora* species, at different universities and research organisations. However, in order to more effectively control plant diseases such as phytophthora in a range of different areas, a higher level of collaboration between the various research and extension providers is needed.

In an effort to indicate what research has been conducted on phytophthora in Thailand and to foster further collaborations we have listed the most recent research reports (Table 4.3.4) and research theses (Table 4.3.5).

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Figure 4.3.2 Screening of durian leaves for resistance to *Phytophthora palmivora*.

Table 4.3.4 Research papers listed in annual reports of the Division of Plant Pathology and Microbiology Department of Agriculture, Bangkok

Year	Title	Author
1994	The study on resistant rootstock of avocado root rot caused by <i>Phytophthora cinnamomi</i> .	Phavakul, K., Kraturisha, C., Koariyakul, S. and Tossapol, M.
1993	Studies on diseases of aloe	Chingduang, S. and Silayoy, E.
1993	Black rot disease of vanilla.	Chingduang, S., Silayoy, E., Likhitearaj, S. and Sasipalin, S.
1993	Selection of some durian rootstocks for their resistance to phytophthora root and stem rot.	Kraturisha, C., Vichitrananda, S., Pingkusol, S. and Leelasettakul, K.
1989	Study on disease of betel vine.	Krengpiem, P., Silayoy, E., Chingduang, S. and Raktham, S.
1989	Study on the causal organism of foot and root rot disease of black pepper.	Krengpiem, P., E. Silayoy, S. Khingduang, S. Raktham and K. Leelasettakul.
1988	Study on varietal reaction of black pepper to foot rot disease under field condition.	Silayoy, E., Leelasettakul, K., Krengpiem, P., Tummakate, A., Kraturisha, C. and Suksawat, S.

Table 4.3.5 Theses, Department of Plant Pathology, Kasetsart University, Bangkok.

Thesis title	Author	Year
Genetics of the resistance to <i>Phytophthora sojae</i> in soybean (<i>Glycine max</i> (L.) Merrill).	Sriphacet, S.	2002
Influence of organic fertiliser from the glutamic acid fermentation on <i>Phytophthora parasitica</i> (Dastur.) and other moulds in tangerine orchard soil.	Phonyangsong, P.	2000
Screening for local durian in southern Thailand resistance to <i>Phytophthora palmivora</i> (Butl.) Butl. by pathogenicity test and isozyme.	Bunjuerdpradit, B.	1999
Efficacy of antagonistic microorganisms for the protection of tangerine root rot caused by <i>Phytophthora parasitica</i> (Dastur.).	Kitjaideaw, A.	1998
Application of <i>Trichoderma harzianum</i> to control root rot of durian caused by <i>Phytophthora palmivora</i> (Butl.) Butl.	Roungwiset, K.	1997
Application of an antagonistic microorganism for the control of root rot of tangerine caused by <i>Phytophthora parasitica</i> (Dastur.).	Seemadua, S	1997
<i>Phytophthora</i> disease or rubber (<i>Hevea brasiliensis</i> Muell.-Arg.): identification, clonal reaction and some chemical control	Srisa-arn, P.	1995
Selection and application of antagonistic microorganisms to control root and stem rot of durian caused by <i>Phytophthora palmivora</i> (Butl.) Butl	Awarun, S.	1994
Effects of antagonistic microorganism used in combination with organic fertilizer and fungicides on root rot of tangerine caused by <i>Phytophthora parasitica</i> (Dastur.).	Intasorn, S.	1994
<i>Phytophthora</i> : identification and detection of fungicide resistance by electrophoresis.	Jamjanya, S.	1994
Fungitoxicity of systemic fungicides and their control efficacy against phytophthora rot and foot rot of tangerine.	Plongbunchong, T.	1992
Studies on tissue culture derived potato plant and callus for resistance to culture filtrate of <i>Phytophthora infestans</i> (Mont.) de Bary and biological control.	Sanyong, S.	1992
Black pod rot of cocoa (<i>Theobroma cacao</i> L.) caused by <i>Phytophthora palmivora</i> (Butl.) Butl.	Kasaempong, Y.	1991
Nutritional status in leaves of durian cv. Mon Thong infected with different levels of <i>Phytophthora palmivora</i> (Butl.) Butl.	Udomsriyothin, T.	1991
Efficacy of mono-dipotassium phosphite against <i>Phytophthora palmivora</i> (Butl.) Butl. on durian.	Bunyanupapong, K	1990
Influence of soil microorganisms on tangerine root rot caused by <i>Phytophthora parasitica</i> (Dastur.).	Termkietpisarn, A.	1990
Epidemiology and chemical preventive control of <i>Phytophthora</i> root and foot rot of tangerine at Rangsit irrigated area.	Wichiencharoen, A.	1990

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4.4 Phytophthora Diseases in Vietnam

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Abstract

Phytophthora diseases have been reported from a range of crops in Vietnam. This chapter provides an overview of the *Phytophthora* species identified and the relative importance of phytophthora diseases on a range of crops including tomato, potato, pineapple, taro, durian, citrus, plum and rubber.

Introduction

Vietnam is a country with two distinct climatic regions: the subtropical region north of the Haivan Mountains, which has four distinct seasons, and the tropical region to the south, which has only two seasons, wet and dry. The presence of mountain ranges in central and northern Vietnam further increases the variety of climatic regions, allowing for a wide range of different plant species to be grown. The subtropical climate in the north, bordering on mountain ranges, allows the growth of tropical and temperate plants in areas close to each other. Various regions in Vietnam also provide an ideal climate for *Phytophthora* species to flourish, and the genus *Phytophthora* is responsible for extensive economic damage in a wide range of different crops throughout the country, including fruit, vegetables, tree plantations and other agricultural crops.

Phytophthora pathogens have been reported to cause leaf blights, stem cankers, heart rots, fruit rots and root rots in a wide range of plant species. However, information on the occurrence and distribution of the various *Phytophthora* species present in Vietnam, disease transmission and progression, and suitable control methods is lacking. A strategic approach to the future study and control of phytophthora diseases is needed.

Distribution of *Phytophthora* in Vietnam

The main information concerning the presence and distribution of phytophthora disease comes from surveys conducted by the National Institute of Plant Protection in Hanoi (NIPP) as part of a national survey of plant diseases. The information from surveys conducted in 1977–1980 has been published in the list of plant diseases in southern Vietnam and the results of a 1997–1998 survey of diseases on fruit crops was published in 1999 (Dang and Ha 1999). The information collected about phytophthora is reproduced in Table 4.4.1.

From these surveys and other field studies, 13 species of *Phytophthora* have been identified in Vietnam. Considering the array of *Phytophthora* species identified in other countries in the region, it is to be expected that many more will be identified in Vietnam. This is especially so, given the current rapid increase in the number of different food, fruit and industrial crops being grown throughout Vietnam. An increase in expertise in plant pathology and diagnostic capability is likely to further increase the number of species identified.

Tomato and Potato

Late blight of tomato and potato is the major disease of these crops. It has been studied in the Red River Delta area since the 1960s. The causative agent is *Phytophthora infestans* and the disease occurs annually from December to March when climatic conditions are cool and humid. All tomato varieties are susceptible to the disease, and infection generally results in a 30–70% yield loss. In severe

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Table 4.4.1 *Phytophthora* species found in Vietnam.

Species	Host	Common name	Diseases	Year	Reference
<i>P. botryosa</i>	<i>Hevea brasiliensis</i>	rubber	leaf fall black stripe	1961	Dang Vu Thi Thanh and Ha Minh Trung (1999)
<i>P. cactorum</i>	<i>Prunus salicilis</i>	plum	fruit rot	1996	Dang Vu Thi Thanh and Ha Minh Trung (1999)
<i>P. capsici</i>	<i>Piper nigrum</i>	black pepper	foot rot	1998	Nguyen Viet Trong (2002)
<i>P. cinnamomi</i>	<i>Ananas comosus</i>	pineapple	heart rot	2001	Dang Vu Thi Thanh et al. (this paper)
<i>P. citrophthora</i>	Citrus species	citrus	fruit rot stem canker		Roger (1951)
<i>P. colocasiae</i>	<i>Colocasia esculenta</i>	taro	leaf blight	1951	Roger (1951)
<i>P. infestans</i>	<i>Solanum tuberosum</i> <i>Lycopersicon esculentum</i> <i>Solanum melongena</i>	potato tomato eggplant	late blight late blight late blight late blight	1951	Roger (1951)
<i>P. nicotianae</i>	<i>Ananas comosus</i> <i>Nicotiana tabacum</i> Citrus species	pineapple tobacco citrus	heart rot black shank stem canker	2001 1967 2002	Dang Vu Thi Thanh et al. (this paper) NIPP (1975) Dang Vu Thi Thanh et al. (this paper)
<i>P. palmivora</i>	<i>Durio zibethinus</i> <i>Cocos nucifera</i> <i>Theobroma cacao</i> <i>Hevea brasiliensis</i>	durian coconut cocoa rubber	leaf blight stem canker fruit rot leaf blight black tripe leaf fall	2000 2002 2002 1965	Dang Vu Thi Thanh et al. (this paper) Dang Vu Thi Thanh et al. (this paper) Dang Vu Thi Thanh et al. (this paper) Dang Vu Thi Thanh and Ha Minh Trung (1999)
<i>P. durian</i>	<i>Durio zibethinus</i>	durian	canker	2002	Dang Vu Thi Thanh et al. (this paper)
<i>Phytophthora</i> sp.	<i>Hevea brasiliensis</i>	rubber	stem canker	1965	Dang Vu Thi Thanh and Ha Minh Trung (1999)
<i>Phytophthora</i> sp.	<i>Zizyphus mauritania</i>	zizyphus	fruit rot	1997	Dang Vu Thi Thanh and Ha Minh Trung (1999)
<i>Phytophthora</i> sp.	<i>Dimocarpus longan</i>	longan	fruit rot leaf blight	1995	Dang Vu Thi Thanh and Ha Minh Trung (1999)

cases, the crop is totally destroyed (Vu 1973). It was noted that the incidence of disease was higher than average in areas with clay soils. Late blight in potatoes and tomatoes in the Red River Delta is controlled by application of a 1% Bordeaux spray every 7–10 days to prevent infection of the crop. The protectant fungicides, Maneb and Zineb at 0.2–0.3% a.i. have also shown a high efficacy against *P. infestans*.

In recent years crop losses in tomato due to late blight have been reduced in the north of Vietnam through the combined use of fungicide applications hybrid varieties with partial resistance to *P. infestans*. In the provinces of Hanoi, Hatay and Vinhphuc, local farmers use an extreme regime of fungicide application in an attempt to control *P. infestans* in tomato. Fungicides such as Zineb and Ridomil are applied at concentrations 2–3 times above the recommended level, and successive spraying is carried out at short time intervals, in some cases every 3–5 days (Ha Minh et al. 2002). Air and water samples taken from the immediate area were found to be contaminated with fungicides. The residue levels in many of these sprayed crops were above the legal limit, making the tomatoes unsuitable for human consumption.

The tomato industry of the provinces of the Red River Delta, such as Hanoi and Haiphong, has potential for expansion in the near future if demand from an increasingly affluent population and the demands from the food-processing industry are to be met. For this expansion to occur, and to safeguard human health, the environment and the future of the tomato industry, the Vietnamese Government needs to develop and implement a cohesive plan for the control of phytophthora diseases in the Delta. Part of this plan should include the education of local farmers in the correct dosage and application of fungicides, and in other disease management tools, in an effort to control late blight.

Taro

Leaf blight caused by *Phytophthora colocasiae* is the major disease of taro in northern Vietnam. The disease was first recorded by Roger (1951). Warm temperatures (24–30°C) and high humidities are required for disease spread, conditions that are found throughout that part of the country. The disease occurs annually, starting between April and May and reaching a peak in July and August when temperatures are a steady 27–29°C and the average monthly rainfall is in the range 201–308 mm. Disease surveys have found leaf blight of taro in all ecological zones of northern Vietnam, with an

average disease incidence of between 21 and 66% (Table 4.4.2).

Phytophthora colocasiae attacks both species of taro grown in Vietnam, *Colocasia esculenta* var. *antiquorum* and *C. esculenta* var. *esculenta*. The level of genetic diversity in *P. colocasiae* was studied using isozymes. Two genotypes were identified from five isolates. Additional RAPD analysis revealed differences between the genotypes found in Vietnam and strains found in other ASEAN countries (Nguyen Van Viet et al. 2002).

Table 4.4.2 The effect of *Phytophthora colocasiae* on taro in northern Vietnam, 2000–2001.

Location	Region	Disease incidence (%)	
		July 2000	July 2001
Kyson	Hoabinh	30	30
Vinhtuong	Vinhphuc	52	53
Hoaiduc	Hatay	30	31
Tuliem	Hanoi	35	31
Dongtrieu	Quangninh	47	66
Dongson	Thanhhoa	20	24

Source: Nguyen et al. (2002)

Pineapple

Pineapple has become an increasingly important crop in Vietnam. In 2001, pineapples covered 32,000 ha of agricultural land, with government targets for the year 2010 set at 50,000 ha. Heart-rot disease is one of the major causes of losses in Vietnam's pineapple crop. The disease has been found in all pineapple-growing areas in the northern and central regions of the country, including Thuathien-Hue, Nghean, Hatay, Bacgiang, Thanhhoa and Ninhbinh. The pineapple variety Cayenne appears to be more susceptible to heart rot than other varieties. The disease incidence 2 months after cultivation in plantations in the Quang Nam region was 35%. After 3 months, it had risen to 60% in some plantations. Interestingly, in regions with very low soil pH levels (3.5–4.2) such as Tiengiang province and Ho Chi Minh City, heart rot disease has not been found (Table 4.4.3). However, it is unclear whether this is due to low soil pH levels or other factors.

A survey in the Donggiao Ninhbinh region revealed that 40% of the samples taken yielded *Phytophthora* after incubating leaf material on potato sucrose agar (PSA). Further identification of the strains obtained revealed the presence of both *P. cinnamomi* and *P. nicotianae* (Table 4.4.4). Samples taken from Hatrung–Thanhhoa in June 2002 were also infected with *P. cinnamomi*. To confirm that *P. nicotianae* and *P. cinnamomi* are responsible for heart-rot disease in

pineapple, glass house trials were conducted at NIPP in 2001. Fifteen days after inoculation with both *Phytophthora* species, 100% of plants displayed symptoms of heart-rot disease (Ngo et al. 2001). Application of 4% Phosacide 200 (a phosphonate source) and 0.25% Aliette 80WP (Rhône Poulenc) reduced the incidence of disease by around 96%.

In field trials at Donggiao Ninhbinh, seedlings dipped in fungicide solution before planting showed a reduced level of infection 45 days after treatment (Table 4.4.5).

Phytophthora heart rot causes significant problems in pineapple cultivation in Vietnam. Continued research into disease development in the field, and integrated disease management through cultivar selection, drainage, cultivation, fungicide application and alternative methods, is needed to establish effective integrated disease management strategies for pineapple cultivation that will allow for the continued and successful expansion of the industry.

Citrus

Phytophthora citrophthora was first recorded on oranges in the Mekong Delta in the 1950s, and was not observed again until the 1970s, when it was found on orange in northern and central Vietnam.

The pathogen has since spread significantly and it now affects fruit in all citrus-growing areas, such as the Thanh tra area of Thua Thien Hue, and Ninhbinh in Tien Giang.

P. citrophthora attacks the stem and fruit, resulting in gummosis and fruit rot symptoms. The disease develops quickly in the rainy season, and is most severe in July and August. In March 2002, disease incidence in orange in Caophong–Hoabinh was 10% but had risen to 20–30% by August. Mandarin was more severely affected, with some orchards suffering total crop loss and the death of many plants. Samples taken from plants suffering citrus stem canker in the Tien Giang province were identified as *P. nicotianae* (A. Drenth, unpublished data).

Phytophthora diseases on citrus have been studied only sporadically in Vietnam, and have often been limited to surveys of disease incidence and severity. There has been no research into the development of control strategies for the disease, nursery management, the breeding of resistant varieties and the use of resistant rootstock. New citrus plantations have been established in Hagiang, Tuyenquang and Vinh Long, and the area devoted to citrus continues to increase. Research into phytophthora diseases and their control is required now to safeguard the future of the industry.

Table 4.4.3 Influence of soil pH on incidence of heart-rot disease, 2000–2001.

Location	Region	Soil pH	Total number of plants surveyed	Disease incidence
Donggiao	Ninhbinh	5.7–7.9	960	211 (21.9%)
Le minh Xuan State farm	Ho Chi Minh City	3.5–4.1	880	0
Tanlap State farm	Tien Giang	3.5–4.2	750	0

Table 4.4.4 Identification^a of *Phytophthora* species causing heart-rot disease in pineapple.

Location	Year	Number of samples	<i>P. nicotianae</i> infected samples	<i>P. cinnamomi</i> infected samples
Donggiao	2001	86	28 (32.5%)	6 (7%)
Hatrung	2002	2	–	2 (100%)

^a Identifications by Dr André Drenth, University of Queensland, Australia, August 2001 and June 2002.)

Table 4.4.5 Effect of fungicide on phytophthora heart rot in pineapple in Donggiao, Vietnam, August 2001.

Treatment	Total number of plants tested	Disease incidence
0.25% Aliette 80WP	240	11 (4.6%)
4% Phosacide 200	240	12 (5.6%)
Control	240	47 (19.6%)

Durian

Durian (*Durio zibethinus* Murr) is one of most favoured fruit crops in southern Vietnam. In recent years, the durian-growing area has rapidly expanded north to the southern and central highlands, displacing rice and other crops due to higher profitability that can be obtained from cultivating durian.

P. palmivora causes a wide range of diseases in durian, including root rot, stem canker, fruit rot and leaf blight. It has been found in all durian growing areas of the southern and central highlands. In 2001, the disease also affected durian growing in the lowlands, and was particularly severe in Quang Nam province. Of the 3075 plants growing in Que Trung commune, 2138 were killed by *P. palmivora* at an economic loss of 15 billion VND (USD1.5 million). Elsewhere in the country, the disease was found to be most prevalent in Cai Be, Tien Giang, with 24.6% of plants infected. Disease incidence was related to plant age, with plants more than 10 years old being most susceptible (Table 4.4.6).

An ACIAR-funded study, 'Management of *Phytophthora* disease in durians', was conducted by the University of Melbourne, Australia, Kasetsart University, Thailand, and the Southern Fruit Research Institute of Vietnam. The study identified several orchard-management practices, such as phosphonate trunk injections and improved nursery hygiene, which can be combined into an integrated disease management package specifically tailored to meet the needs of each region. Full details of this study and its outcomes can be found in chapter 8 of this monograph and will therefore not be discussed here.

Plum

In recent years, black spot disease of plum (*Prunus salicilas*) has seriously reduced crop yields in Bac Ha and Moc Chau provinces. *Phytophthora cactorum* was identified as the causal agent. In Bac Ha in March 1996, the disease affected 300 ha of young plum fruit causing serious damage and a 20% yield loss. During 1997 and 1998, the disease was less widespread but the damage caused was more severe, with some gardens showing black spot disease in up to 50% of their crop.

Disease symptoms on plum are typically white--grey water-soaked spots on young fruit, developing into sunken black spots with brown edges as the disease progresses. In cases of severe infection, the whole fruit will shrivel and fall from the tree. The sunken spots may become covered with white mycelium in damp conditions, and *P. cactorum* conidia have been isolated from the spots in these conditions. Sporangia can be isolated and grown into culture on carrot, kidney bean or potato dextrose agar, but sporulation has not been observed in vitro.

In March 1998, the infectivity of *P. cactorum* on plum gardens was studied at Bac Ha. Initial disease symptoms were observed on all treated fruit 3–5 days after inoculation, but in many cases did not develop further and the final incidence of disease was low, probably due to the relatively high temperature (18–25°C) in the field during that time. The results of the 1998 study are summarised in Table 4.4.7.

Late February in northern Vietnam is typically cool and damp, with daytime temperatures of 12–14°C, nights that are around 10°C cooler than the days,

Table 4.4.6 Phytophthora disease incidence in durian growing areas

Location	Region	Number of plants surveyed	Disease incidence (%)			
			Total	< 5 years	6–10 years	>10 years
Que Son	Quang Nam	370	22.0	5.1	7.5	9.4
Long khanh	Dong nai	280	21.1	5.0	6.1	10.0
Cai Be	Tien Giang	182	24.6	3.8	10.4	10.4

Table 4.4.7 Results of inoculation of plum fruit with pure culture of *Phytophthora cactorum* at Bac Ha, March 1998.

Treatment	Number of fruit	Infected fruit	Disease incidence (%)	Time to appearance of symptoms (days)
Control - distilled water	56	0	0	-
<i>P. cactorum</i>	191	7	13.4	3–5

and frequent fog. These conditions are ideal for *P. cactorum* infection. Development of black spot disease on young plums is therefore swift in March and early April, slowing as the temperature rises towards the end of the month. It has been observed that young trees are more susceptible to black spot disease than more mature trees. In March 1998, the disease incidence on 2-year-old plum trees was 10%, while 4-year-old trees suffered only a 2.1% disease incidence.

In 1999, plums on the hills of Bac Ha suffered a widespread outbreak of black spot disease. This allowed for a study of disease distribution in relation to geographical factors. The incidence of disease was found to vary widely according to location on the hill. On 5 March 1999 the disease incidence at the summit of the hill was 0.7%, at the middle of the hill it was 3.1% and in the foothills it was 3.9%. By 20 March the pattern of disease incidence had changed dramatically; at the top of the hill it was 43%, in the middle 81% and in the foothills 26.3% (Table 4.4.8). Very similar results were obtained from a similar survey conducted in the previous year.

The reason for the high disease incidence and severity halfway up the hill is most likely microclimatic factors that lead to differences in humidity and temperature between the different sites.

Rubber

Rubber (*Hevea brasiliensis*) is a highly valuable industrial commodity that is grown all over Vietnam. Rubber plants were imported to Vietnam in 1897 to establish plantations, with 288,000 ha devoted to its growth in 1996 and a government target of 700,000 ha by the year 2005. Most of the large rubber plantations are located in the southern and central highlands of Vietnam.

The 1960s saw the start of studies concerning diseases in rubber in Vietnam. Of the 19 diseases that affect rubber in Vietnam, leaf fall, black stripe and stem canker are caused by *Phytophthora* species. *P. palmivora* has been isolated from around 72% of

rubber plants affected with black-stripe disease, while *P. botryosa* can be found in 75–80% of leaves and fruit suffering from leaf-drop disease. Both *P. palmivora* and *P. botryosa* are known to infect trees in all parts of the country.

In the southern highlands during the wet season, disease incidence of leaf-drop and black-stripe diseases has been as high as 45% and 34%, respectively. Leaf-fall disease is generally more severe in Dong Nai and Binh Long than in Dau Tieng and Tay Ninh. The two diseases combined can reduce rubber production by 23 to 36.8% annually. Of the rubber varieties grown (PR107, PB86, RRIM600, PB310, PR255 and PB244) most are susceptible to black-stripe disease, with only one, PR107, being resistant. The application of Ridomil 72 WP and Difolatan has proven to be effective in controlling the disease. Bark-rot disease, also caused by *Phytophthora* species, is found only in the northern regions of the country.

Conclusions

Phytophthora diseases are responsible for some of Vietnam's major crop losses in tomato, potato, citrus, pineapple, plum, black pepper, rubber, and durian. Identification of *Phytophthora* is currently based on disease symptoms and morphological characteristics. An increased capability is needed to accurately distinguish *phytophthora* diseases from other soil-borne diseases and to be able to identify isolates down to species level.

Very little research on *Phytophthora* and *phytophthora* diseases has been carried out in Vietnam. Progress in the following areas is urgently needed:

- obtaining expertise in and information on disease symptoms and methods to isolate potential *Phytophthora* pathogens
- gaining expertise in the identification of *Phytophthora* pathogens down to species level, including the use of molecular methods for identifying *Phytophthora* species

Table 4.4.8 Development of black spot disease on plum fruit at Bac Ha in March 1999.

Location	5 March		10 March		15 March		20 March	
	DI ^a (%)	DS ^b (%)	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)
Foothills	3.9	1.3	19.5	7.1	11.8	9.3	26.3	16.3
Middle of hill	3.1	1.5	43.6	17.7	49.7	18.8	81.2	46.0
Top of hill	0.7	0.2	25.0	8.0	32.0	8.4	42.6	14.2

^a Disease incidence. ^b Disease severity.

- selection and resistance-screening methods that allow the development of crops resistant to infection by *Phytophthora* species
- information on host spectrum, disease development and control methods of the various *Phytophthora* species
- the development of an integrated pest management approach for the control of phytophthora diseases. This should include the establishment of pilot schemes, and the education and training of farmers and growers, especially about environmental protection.

Collaborative work and exchange of information and knowledge between the scientists of Vietnam, other ASEAN countries and Australia is required if the successful control of *Phytophthora* is to be achieved.

Acknowledgments

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4.5 Phytophthora Diseases in the Philippines

L.A. Portales¹

Abstract

An overview is provided of the phytophthora diseases reported and the species of *Phytophthora* identified in the Philippines. *Phytophthora palmivora* and *P. nicotianae* are the most common species and have caused considerable disease losses in a range of crops of significant economic importance to the Philippines.

Introduction

Stretching 1839 kilometres north-to-south, the Republic of the Philippines has a total land area of 300,000 km² spread over 7107 islands. The Philippines is a tropical country with an average temperature of 32°C (80°F). The months of March to June are hot and dry (36°C), rains and typhoons abound from July to October, while November to February are pleasantly cool (around 23°C) and dry. In mountainous regions, temperatures can dip to about 15°C. The long wet and cool seasons are conducive to the infection of the country's agricultural crops by phytophthora diseases.

Overview of *Phytophthora* Problems in the Philippines

The first crop reported to be infected with *Phytophthora* was coconut in 1908, but comprehensive studies on the disease did not begin until 1919 (Table 4.5.1). Between 1919 and 1933, seven important papers dealing with phytophthora on coconut, citrus, cocoa, eggplant, santol and cinchona were published (Table 4.5.1) The papers reported detailed information on the morphology and pathogenicity of *Phytophthora* species, and the mode of disease propagation, disease symptoms and control measures. From 1934–1971, only three papers reporting phytophthora diseases were published (potato late blight, pineapple heart rot

and eggplant fruit rot), but since 1972 many government research agencies and academic institutions have become involved in phytophthora research.

Economic Importance

Although many species of *Phytophthora* have been detected and are known to cause serious crop losses in the Philippines, published data on the impact of these pathogens is not available for most crops. Disease losses for only four crops have been published, and these losses occurred in isolated areas.

Although the disease is usually of minor importance to eggplant, under favourable conditions and in dense planting, *Phytophthora* may cause serious infection and yield loss. Eggplant in the garden of the College of Agriculture in Los Baños and environs was found to be infected with *Phytophthora*. Disease losses on pineapple were reported in three municipalities of Laguna, namely Los Baños, Calauan and Alaminos. Fifty per cent of the pineapples in a 2 ha field at Alaminos were infected. The outbreak of a serious seedling blight of cinchona was first reported in 1932, from a nursery of the College of Agriculture in Los Baños, Laguna, where 45% of the plants were infected. The 1924 infection of santol (*Sandorium koetjape*) resulted in the death of 90% of infected seedlings, the disease being manifest as blight on the different parts of the young seedlings, causing eventual collapse and decay. Table 4.5.2 presents these crops with the reported level of infection.

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Table 4.5.1 Phytophthora diseases of various crops in the Philippines

Phytophthora species ^a	Host	Common name	Disease	Year first reported	Reference	
<i>P. cactorum</i>	<i>Theobroma cacao</i>	Cocoa	Oriental cocoa pod disease	1916	Mendiola and Espino (1916)	
<i>P. capsici</i>	<i>Piper nigrum</i>	Black pepper	Crown/root rot	1994	Tsao et al. (1994)	
<i>P. citrophthora</i>	<i>Citrus</i> spp.	Citrus	Foot rot	1968	Rosario (1968)	
			Gummosis	1968	Rosario (1968)	
			Brown rot	1974	Quimio and Quimio (1974)	
			Crown/root rot	1994	Tsao et al. (1994)	
			Crown/root rot	1994	Tsao et al. (1994)	
<i>P. colocasiae</i>	<i>Colocasia esculenta</i>	Gabi	Leaf blight	1916	Mendiola and Espino (1916)	
		Santol	Crown/root rot	1994	Tsao et al. (1994)	
<i>P. infestans</i>	<i>Solanum tuberosum</i>	Potato	Late blight	1921	Lee (1921)	
<i>P. meadii</i>	<i>Hevea brasiliensis</i>	Rubber	Black thread	1926	Teodoro (1926)	
<i>P. nicotianae</i>	<i>Piper nigrum</i>	Black pepper	Crown/root rot	1994	Tsao et al. (1994)	
		Citrus	Foot rot	1921	Lee (1921)	
	<i>Solanum melongena</i> ^c		Gummosis	1968	Rosario (1968)	
			Fruit rot	1925	Ocfemia (1925)	
			Phytophthora blight, fruit rot	1974	Quimio and Quimio (1974)	
			Heart rot / pineapple wilt	1962	Quebral et al. (1962)	
			Watermelon	Phytophthora rot	1974	Quimio and Quimio (1974)
	<i>P. palmivora</i>	<i>Persea americana</i>	Avocado	Crown/root rot	1994	Tsao et al. (1994)
		<i>Theobroma cacao</i>	Cocoa	Black rot/stem canker	1979	PCARR (1979)
		<i>Artocarpus camansi</i>	Camansi	Crown/ root rot	1994	Tsao et al. (1994)
		<i>Cinchona calisaya</i>	Cinchona	Seedling blight	1933	Celino (1933)
<i>Citrus</i> spp.		Citrus	Phytophthora blight	1968	Rosario (1968)	
<i>Cocos nucifera</i> ^a		Coconut	Bud rot	1919	Reinking (1919)	
<i>Durio zibethinus</i>		Durian	Crown/root rot	1994	Tsao et al. (1994)	
<i>Artocarpus heterophyllus</i>		Jackfruit	Crown/root rot	1994	Tsao et al. (1994)	
<i>Lansium domesticum</i>		Lanzones	Crown/root rot	1994	Tsao et al. (1994)	
<i>Euphoria longana</i>		Longan	Crown/root rot	1994	Tsao et al. (1994)	
<i>Garcinia mangostana</i>		Mangosteen	Crown/root rot	1994	Tsao et al. (1994)	
<i>Azadirachta indica</i>		Neem	Crown/root rot	1994	Tsao et al. (1994)	
<i>Phalaenopsis</i> spp.		Orchid	Black rot	1968	Ela (1968)	
<i>Carica papaya</i> ^a		Papaya	Fruit rot	1918	Reinking (1919)	
<i>Hevea brasiliensis</i> ^a	Rubber	Black rot of fruit	1918	Reinking (1919)		
<i>Hevea brasiliensis</i> ^a	Rubber	Canker	1926	Teodoro (1926)		
<i>Annona muricata</i>	Soursop	Crown/root rot	1994	Tsao et al. (1994)		
<i>Tamarindus indica</i>	Tamarind	Crown/root rot	1994	Tsao et al. (1994)		
<i>P. phaseoli</i>	<i>Sandoriam koetjape</i>	Santol	Leaf blight	1928	Clara (1928)	

^a The use of these names has been discontinued in the literature: *P. faberi* is the synonym of *P. palmivora*, *P. ommitoora* is the synonym of *P. cactorum*; both *P. melongenae* and *P. terrestris* are synonyms of *P. nicotianae* (= *P. parvisitica*).

Management of Phytophthora Disease in the Philippines

Early studies on phytophthora management and control recommended a mix of cultural management, chemical control and quarantine policy. Control measures included the draining of excess water, cultivation of soil for increased aeration, increasing the space between neighbouring trees, and the pruning of excess branches to improve ventilation and to allow sunlight to enter. Maintaining clean culture by the removal and burning of infected plants and plant parts was also recommended, together with the use of resistant varieties and spraying with Bordeaux mixture.

Table 4.5.2 Reported crops with known *Phytophthora* infection.

Crop	<i>Phytophthora</i> species	Disease	Level of infection (%)
Eggplant	<i>P. nicotianae</i>	Fruit rot	25–75
Pineapple	<i>P. nicotianae</i>	Heart rot	20–50
Cinchona	<i>P. palmivora</i>	Seedling blight	45
Santol	<i>P. phaseoli</i>	Leaf blight	90

Nursery Management²

A survey of the prevalence of phytophthora diseases in the Philippines by the Bureau of Plant Industry resulted in the following recommendations for the management and control of phytophthora in nursery operations.

1. Sterilised soil, sand or other planting media should be used for the germination of seeds and cuttings. Leftover soil, pots and plastic containers should also be sterilised if they are to be re-used.
2. Porous materials with good aeration and drainage properties, such as sand, sawdust, or composted tree bark, should be used instead of pure soil, or in addition to pure soil, whenever possible. Adequate nutrients should be provided by the use of organic and inorganic fertilisers.
3. Clean, sterilised soil should be stored in closed containers such as soil bins, to prevent contamination. Diseased materials, foot traffic, animals, and run-off water may contaminate soil left on bare ground or in uncovered containers.
4. Only clean seeds extracted from healthy fruits should be used. Never use seeds taken from fruit

already on the ground. Avoid using infected fruit or fruits showing lesions or other signs of disease.

5. Use only clean tools and with clean hands. Tools such as shovels, trowels, shears and knives should be washed, dried and sterilised after each use, with either 70% ethanol or 10% bleach.
6. Hoses should not be left on the ground after use.
7. Water sources should be protected from contamination by soil or diseased material. Hands or tools should never be washed in water stored for watering.
8. Seed boxes and potted plants should be kept on raised benches above the ground. Where this is not possible, cover the ground with 5–8 cm of gravel to avoid contamination from standing or splashing water. Low areas in the nursery, where the risk of contamination from standing water is high, should not be used.
9. Infected or diseased plants and plant material should be removed from the propagation area and disposed of appropriately.

Training and Extension

During 1989 and again in 1990–91 the Philippine–German Biological Plant Project and the Deutsche Gesellschaft für Technische Zusammenarbeit (PGBPPP/GTZ) funded visits by Dr Peter H. Tsao, from the University of California in Riverside (UCR) to the Bureau of Plant Industry (BPI) for 1 and 6 months, respectively, to train BPI plant pathologists on aspects of *Phytophthora* and phytophthora diseases.

There were 16 participants in a training course on ‘Detection, isolation and identification of *Phytophthora* diseases in the Philippines’ run by Dr Tsao during January 1991 at the Crop Protection Division of BPI–Manila. They included representatives from eight of the twelve Regional Crop Protection Centers, and six people from the BPI Research Centers, including one from the National Crop Protection Center at Los Baños and one from the Department of Plant Pathology of the University of the Philippines at Los Baños. During 1992, and again in 1993–95, a BPI staff member joined the research team at UCR for further training on the biology and control of phytophthora, and in isolation and identification techniques. Numerous other training courses have been conducted by overseas experts over the past decade. These were typically tied to specific research projects including the outbreak of budrot following the introduction of hybrid coconut (see chapters 6.2 and 6.3).

² See also Chapter 7.1.

Technology Information and Dissemination

As a result of the BPI survey into the prevalence of phytophthora diseases in the Philippines, the booklet 'How to produce healthy plants' was funded and published by BPI-PGBPPP/GTZ (Tsao 1993). The booklet highlights the importance of clean soil, seed and stock, and hygienic nursery practices, in order to run a successful nursery operation.

An easy-to-understand leaflet entitled '*Phytophthora* disease diagnosis' was produced by the Crop Protection Division of BPI. It aimed at increasing growers' awareness of the disease, and contains basic information on disease diagnosis, from isolating and purifying *Phytophthora* from crop samples, through to identification and pathogenicity testing procedures. Similar material has been produced as part of an FAO-funded project on coconut bud rot.

Conclusion

Phytophthora diseases have been detected in the Philippines since the early 1900s. The country's climate and environment mean these diseases have been detected over a large range of crops and geographic locations. All BPI field surveys have recovered *Phytophthora* samples using isolation techniques such as selective agar media and baiting procedures. However, comprehensive information on the impact of phytophthora disease on plant production in the Philippines is lacking. Thus, a concerted effort by research agencies and academic institutions into suitable management and control strategies for the disease is needed, so as to minimise and manage crop losses.

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5 Isolation of *Phytophthora* from Infected Plant Tissue and Soil, and Principles of Species Identification

André Drenth and Barbara Sendall¹

Abstract

In order to assign the cause of a disease or disorder to a particular pathogenic organism it is important that the causative agent be identified, and that additional pathogenicity tests are conducted to show beyond reasonable doubt that the organism in question can indeed cause the disease. Although the isolation of *Phytophthora* pathogens is not difficult it is different to the isolation and identification of many true fungi. We give an overview of media, antibiotics and methods available that may be used for isolation and identification of *Phytophthora* species in the tropics.

Introduction

It is estimated that *Phytophthora* species cause 90% of the crown rots of woody plants. However, lack of knowledge on how to isolate *Phytophthora* often leads to negative results and hence other pathogens such as *Fusarium*, *Pythium*, *Rhizoctonia* and nematodes are frequently blamed for root and crown rots (Tsao 1990). Unlike species of *Pythium* and *Fusarium*, which are generally associated as saprophytes or opportunists with plants and soil, *Phytophthora* species associated with diseased plants are likely to be the causal agent of the disease. This is because most *Phytophthora* species attack only living or freshly wounded tissue. They are primary invaders and hence do not colonise plant tissue already invaded by other microorganisms. Detection and/or isolation of *Phytophthora* from plant tissue is relatively simple and successful if the tissue is fresh and recently infected. Isolation of *Phytophthora* from necrotic plant tissue is more difficult, because most species of *Phytophthora* have poor saprophytic capabilities, and there may be very little mycelia remaining once the host tissue dies and secondary invaders move in. In addition,

dormant propagules such as chlamydospores and oospores are slow to germinate and emerge from senescent plant tissue. Isolation of *Phytophthora* directly from soil is difficult, but the use of baiting techniques markedly increases the frequency of successful isolation of *Phytophthora* from infested soils.

Isolation Media

The Oomycetes are not true fungi (see Chapter 3.1), and therefore special techniques are required for their isolation. Most species of *Phytophthora* grow rather slowly in vitro compared with saprophytic fungi and bacteria. In addition, bacterial populations need to be kept low because they may suppress the growth of *Phytophthora* by direct competition, by antagonism caused by antibiotic production, or by direct parasitism. The use of selective media usually overcomes these problems. Antibiotics are added to isolation media in order to suppress the growth of bacteria. Also, because *Phytophthora* spp. are out-competed by many fungi, it is desirable to choose media that are nutritionally 'weak'. This reduces the growth rate of fungal contaminants, allowing colonies of *Phytophthora* to become established.

Cornmeal agar (CMA) is the most frequently used basic medium for isolation of *Phytophthora* from

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infected plant tissue. However, other desirable basal media include water agar, and 2% and 4% (v/v) V8 juice agar. Alternatives to these media made with locally available ingredients are cocoa pulp, taro, coconut milk, and carrot mixed with agar-agar.

Selective Media for Isolation from Diseased Tissue

Various media containing different antibiotics and antifungal components can be used to isolate *Phytophthora*. Corn meal agar (CMA) at 1.7% is the most common medium used as a basis. 3-P (Eckert and Tsao 1960; Eckert 1962) (Table 5.1) is suitable for the isolation of *Phytophthora* from freshly diseased tissue but not from old, decayed tissue or freshly infested soil in which the propagules are likely to be spores. This is because high levels of pimaricin can inhibit spore germination. A suitable medium for isolating *Phytophthora* from old plant tissue or soil is 3-P medium + 10 mg/mL pimaricin (Table 5.1). Plates of selective media used for isolations should not contain any free water or condensation on the lids, as water encourages the growth and spread of bacterial contaminants. Ideally, selective media containing antibiotics should be made fresh before use. Otherwise, they should be used within 2–4 weeks of preparation.

Hymexazol-25 and Hymexazol-50 (Masago et al. 1977) contain the fungicide Hymexazol (Tachigaren). This fungicide has been found to suppress most *Pythium* spp. except for *P. irregulare* and *P. vexans*. It can also inhibit some *Phytophthora* spp., including *P. cinnamomi*, *P. citrophthora* and *P. palmivora*. P10VP (Tsao and Ocana 1969) is suitable for isolating *Phytophthora* from soil and infected plant tissue. Hymexazol can also be added to a final concentration of 25–50 mg/mL. P10ARP

(Kannwischer and Mitchell 1978) and P5ARP (Papavizas et al. 1981; Jeffers and Martin 1986) are the media of choice for isolating most species of *Phytophthora* (Table 5.1).

Since the availability of media and antibiotics varies between locations a series of common antibiotics and antifungal and alternative compounds which may be used to produce media suitable for the isolation of *Phytophthora* are given in Table 5.2. In some cases, when samples are relatively clean and secondary invaders are still absent, one can also isolate directly onto media without the use of antibiotics. Infected fruit can be processed in this manner as the *Phytophthora* typically grow quite deeply in the tissue which allows one to cut away the outer part and directly place fruit tissue containing *Phytophthora* mycelium onto agar with a very high success rate.

Isolation of *Phytophthora* from Infected Plant Material

Phytophthora species attack only healthy plant material, including roots. Thus, the pathogen can be present when no symptoms are obvious. *Phytophthora* species are difficult to isolate from necrotic tissue because the tissue often harbours many secondary pathogens. Successful isolation of *Phytophthora* species from diseased tissue involves careful selection of freshly infected tissue. Therefore, it is best to obtain material from the edge of an actively growing lesion. Leaf and stem tissue selected for isolation should ideally contain part diseased and part healthy tissue. Once the tissue has been surface-sterilised, it should be transferred to the appropriate selective medium, and the plates examined regularly for the slow emergence of non-septate hyphae.

Table 5.1 Amount of antibiotic/fungicide required for various *Phytophthora* selective media

Antibiotic/ fungicide	Stock (mg/mL)	Final antibiotic/fungicide concentration (µg/mL)							
		3-P	3-P + 10 µg/mL pimaricin	P ₁₀ VP	P ₁₀ ARP	P ₅ ARP	Hymexazol 25	Hymexazol 50	
Ampicillin	100					250	250	500	500
Benomyl	Powder							10	5
Hymexazol	50							1	1
Nystatin	100							25	25
PCNB	Powder			100	100	100		25	25
Penicillin	50	50	50						
Pimaricin	25	100	10	10	10	5			
Polymixin B	50	50	50						
Rifampicin	10					10	10	10	10
Vancomycin	100			200					

Pythium spp. are almost invariably present on both healthy and diseased roots, crowns and lower stems of plants. There are three ways in which contamination of isolation media by *Pythium* can be minimised:

1. *Pythium* is confined to roots or badly rotted lower stems — choose other parts if possible.
2. *Pythium* is confined to the outer cortex of the root — surface sterilisation will usually kill it; alternatively choose the centre of the root.
3. Hymexazol will inhibit most species, except for *P. irregulare* and *P. vexans*. Care must be taken, however, as it can also inhibit some *Phytophthora* spp., including *P. cinnamomi*, *P. citrophthora* and *P. palmivora*. When these species are suspected, it is wise to use selective media with and without hymexazol.

Preparation and Surface Sterilisation of Tissue

It is important to use aseptic techniques, including flame sterilisation and wiping areas with 70% ethanol, when attempting to isolate *Phytophthora* from infected plant tissue. Place well-washed roots, stems or leaves suspected to be infected with *Phytophthora* into a shallow layer of distilled water. Leave for 24–48 hours in the light, at 18–25°C and examine for sporangial development. If sporangia are found, a small infected plant piece can be cut off, surface sterilised and transferred to selective media.

Infected fruit is easily treated by cutting off the outer parts and placing small pieces of the freshly cut fruit onto selective media. Leaf tissue which is reasonably clean may be placed immediately onto selective media but it is almost always better to surface sterilise it first. Surface sterilise leaf and stem tissue by dipping in 70% ethanol for 30–60 seconds. Blot tissue dry between sterile filter paper before placing on selective media. If wet plant material is placed onto media, bacteria can grow rapidly and suppress the growth of *Phytophthora*. If the stems are particularly thick (0.5–1 cm wide), they can be dipped in 70% ethanol for 10–30 seconds, and then quickly flamed to burn off the excess ethanol. Small sections can then be taken either side of the lesion, and embedded directly into selective media.

Diseased roots often need more preparation. Place the roots in a beaker and wash them in gently running water for several hours. This process removes the bacteria and stimulates production of sporangia. After washing, cut out small sections of advancing root lesions, surface-sterilise and blot the roots dry between sterile filter paper. Transfer to

selective media. Infected root material can be surface sterilised by using either one of the two methods below: (i) dip pieces of root tissue in 70% v/v ethanol for about 1 minute, wash for 10–20 seconds in sterile distilled water and blot dry on sterile filter paper or tissue paper. Cut root pieces into 0.5 cm lengths before placing onto selective media; (ii) dip root tissue in a 1:10 dilution of commercial bleach (sodium hypochlorite; approx. 0.5% v/v final concentration) for about 30 seconds. Rinse the roots in sterile water and blot dry on sterile filter paper. Cut root pieces into 0.5 cm lengths before placing onto selective media. Sterilisation with ethanol results in fewer problems with bacterial contamination and gives good recovery of most species of *Phytophthora*. It is also important that root pieces are very well dried by blotting and pushed just under the surface of agar instead of just being placed on top. This will ensure good contact between bacteria in the tissue and the antibiotics in the media. *Phytophthora* species will grow through the media quickly leaving bacterial contaminants behind.

Biology of *Phytophthora* from Plant Tissue and Soil

Phytophthora can also be isolated from infected plant tissue or soil by baiting. This method is useful for two reasons: (i) the initial steps can be performed in the field, and (ii) surface sterilisation of the baited tissue is usually not required.

The best way to go about sampling soil for *Phytophthora* is as follows: where possible, samples should be taken from moist soil, near healthy roots at least 5 cm below the soil surface. The soil surface is often dry and heated by the sun, making it an inhospitable place for *Phytophthora*. Soil samples are often best taken during or immediately after wet weather, which typically increases *Phytophthora* activity. Sampling is often best under the edge of the plant/tree canopy, as root growth is more vigorous there than immediately adjacent to the stem.

Samples should be handled carefully after collection. If soil samples are exposed to drying or high temperatures (+45°C) they will lose their viability. Therefore, samples should not be left in an enclosed vehicle in warm weather. Place your soil samples in plastic bags to prevent drying out and put them in an insulated icebox to prevent overheating. Avoid low temperatures too, as *Phytophthora* does not withstand freezing. In case the samples need to be stored, do not use a refrigerator but hold them at 10–15°C and ensure that the samples are moist (add water if the samples are dry).

It is best to process samples within a few days but soil samples can be kept like this for a few months. If soil samples dry out during storage, they can be re-moistened for 1–7 days before isolation is attempted. This can stimulate production of sporangia or germination of chlamydospores or oospores.

Many plant parts can be used to selectively bait a target species of *Phytophthora*. These include fruits, seeds, seedpods, seedlings, cotyledons, leaves, leaf discs/strips, and petals.

There are three main baiting techniques:

- insertion of soil or infected tissue into a hole made on a fleshy fruit (e.g. apple, cocoa pod, pear, watermelon) — a large fruit is desirable
- planting seeds, seedlings or rooted cuttings into field soil followed by heavy watering to induce infection
- floating or partial immersing baits of various types in a water and soil mixture, which is the most widely used method for isolating *Phytophthora* spp.

The choice of bait is dependent on the species of *Phytophthora* that is suspected to be the causal agent of disease, and the host plant. A list of baiting techniques is provided in Table 5.3.

The following method is described by Chee and Foong (1968). Core out 8 mm diameter plugs of tissue from a green (unripe) cocoa pod. Insert a wedge of diseased tissue (1 cm wide × 2 cm long) or soil into the hole and push it in so that the end is flush with the outside of the fruit. Alternatively, the pod can be cut at an angle and very fine pieces of tissue such as bark inserted into the cuts. Seal the pod in a plastic bag and incubate at room temperature. Up to six wedges can be inserted into a single pod. After 4–5 days, brown discolouration should be obvious around the plugs. A firm rot indicates the presence of *Phytophthora*, a soft rot the presence of saprophytic organisms. Take a small amount of healthy tissue from around the discoloured patch. If the tissue is taken from inside the pod, it does not require surface sterilisation. Plate tissue pieces onto selective media. Other baits such as papaya and apple may also be used if cocoa pods are not available.

For those techniques requiring partial immersion of baits in soil, or floating of baits in soil, high water:soil ratios (4:1 or greater) are desirable. It is best to use distilled or deionised water or some other source of water such as bottled drinking water free from chloride or copper ions. Dilution of the soil may also dilute inhibitors present in the soil, enhancing the

formation of sporangia and zoospores. Isolations from infected bait material should be made from healthy tissue surrounding lesions. In the case of leaf discs/strips or petals, the entire tissue may be placed on the media. Include a control of water only to ensure water or baits are not infested.

Culturing and Storage of *Phytophthora*

Culturing

Most *Phytophthora* species grow well on a range of media. Cultures of *Phytophthora* should be grown at 15–25°C in a dark incubator. Cultures should be transferred every 2–4 weeks to maintain vigour. For long-term storage, water storage as described below is recommended. The pathogenicity of *Phytophthora* cultures is known to decrease after prolonged storage on media. In case pathogenicity studies need to be performed, serial passage through the host plant is required. Another alternative is storage of cultures in liquid nitrogen, which seems to overcome the problem of loss of pathogenicity.

Long-term storage in sterile water

Phytophthora strains should be maintained as living cultures for two reasons: (i) to provide reference strains for various studies involving pathogenicity, virulence, mating type etc. and (ii) as a source of DNA for genetic diversity and evolutionary studies.

To store cultures of *Phytophthora*, cut 8–10 small blocks from the edge of an actively growing colony culture, and place in small, screw-capped glass bottles containing autoclaved distilled water. The caps should be tightened during storage and the vials placed at room temperature in the dark. Most species of *Phytophthora* can be stored this way but the isolates will lose pathogenicity and aggressiveness during storage and cannot be used for studies in that area after prolonged storage. Ideally, cultures should be revitalised once a year or every second year. For some species, a soybean or maize seed can be added before autoclaving the water as it seems to induce oospore formation in homothallic species. Record details such accession number, identification, date, host, locality, identifier's name etc.

Identification of *Phytophthora*

The genus *Phytophthora* has been widely acknowledged as taxonomically 'difficult' (Brasier 1983) as many of the characters used for species identification are plastic, highly influenced by environment, show overlap between species, and

have an unknown genetic basis. Nonetheless, since a major review of the genus was performed by Waterhouse (1963), morphological characters have remained the basis for species identification and taxonomy (Newhook et al. 1978; Stamps et al. 1990). Waterhouse classified species based primarily on papillation and caducity (easy detachment) of sporangia, type of antheridial attachment, and mating system. Based on this analysis, the genus was divided into 6 major groups (Table 5.4), which were intended solely as an aid to species identification, and were not meant to imply a natural classification (Waterhouse 1963).

Many species of *Phytophthora* can be easily identified. However, the morphological differences among some species are few and variable, making it difficult to classify them accurately. Identification of *Phytophthora* is based on the taxonomic keys of Waterhouse (1963) and Stamps et al. (1990). Characteristics that are used to classify species of *Phytophthora* include sporangium morphology, morphology of sexual structures such as antheridia, oogonia and oospores, presence or absence of chlamydospores, and morphology of hyphae.

Cultures

It is important to remember that, on selective media, most *Phytophthora* species will not sporulate and form characteristic propagules for identification. Therefore, cultures should be incubated at the optimum temperature for the species suspected, on a natural medium such as V8 juice, carrot agar or Lima bean agar. In order to identify an isolate of *Phytophthora* to species level, it is necessary to induce the production of asexual and sexual structures that will aid in species identification. Characteristics of the mycelium, and whether the culture produces chlamydospores, will also assist in identification.

Morphological characters

There are a number of morphological characters upon which identification of *Phytophthora* species is based. These include sporangium shape, papillation, and caducity, sporangiophore morphology,

presence of chlamydospores and hyphal swellings, antheridial attachment, and whether sexual reproduction is heterothallic or homothallic.

Sporangia

Sporulation in *Phytophthora* cultures provides important clues for species identification. Important characters to observe are:

- sporangium morphology (shape, size, length:width ratio) papillation of the sporangium caducity (shedding of the sporangium at maturity) (Figure 5.1)
- length of the pedicel on the sporangium
- proliferation of sporangium (production of new sporangium within a sporangium that has germinated directly)
- branching of the sporangiophores on which the sporangia are borne.

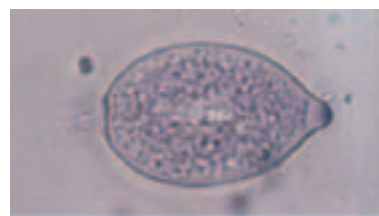


Figure 5.1 *Phytophthora palmivora* sporangia, papillate, caducous and with a short pedicel.

Some species of *Phytophthora* produce sporangia readily on the surface of agar media. However, many species need to be cultured in water, mineral salt solutions or dilute soil extracts before they will produce sporangia. It is important to remember that sporangia production in *Phytophthora* is dependent on light (Schmitthenner and Bhat 1994). Table 5.5 provides a general guide to which species of *Phytophthora* produce sporangia on agar media.

Sporangia can be induced by cutting blocks of 0.5 cm² agar discs from the edge of a colony that has been grown on V8 juice agar or carrot agar. Cultures 2–4 days old are most suitable. Incubate the discs in

Table 5.4 Classification of *Phytophthora* into six groups by Waterhouse (1963).

Group	Sporangia	Antheridial attachment	Examples
I	papillate	paragynous	<i>P. cactorum</i> , <i>P. clandestina</i>
II	papillate	amphigynous	<i>P. capsici</i> , <i>P. palmivora</i>
III	semi-papillate	paragynous	<i>P. inflata</i> , <i>P. multivesiculata</i>
IV	semi-papillate	amphigynous	<i>P. infestans</i> , <i>P. ilicis</i>
V	non-papillate	paragynous	<i>P. megasperma</i> , <i>P. sojae</i>
VI	non-papillate	amphigynous	<i>P. cinnamomi</i> , <i>P. drechsleri</i>

a shallow layer of distilled water (or pond water or salt solution or soil extract) in a Petri dish, at room temperature (22–24°C). Incubation under continuous fluorescent light is recommended. Sporangia are produced within 12 hours in some species, and typically within 1–2 days.

Table 5.5 *Phytophthora* species that produce sporangia on solid or liquid media.

Sporangia produced on agar	Sporangia produced in liquid media
<i>P. capsici</i>	<i>P. cambivora</i>
<i>P. heveae</i>	<i>P. cinnamomi</i>
<i>P. megakarya</i>	<i>P. citricola</i>
<i>P. nicotianae</i>	<i>P. cryptogea</i>
<i>P. palmivora</i>	<i>P. drechsleri</i>

Chlamydospores and hyphal swellings

Chlamydospores are thick-walled spores that function as a resting spore. They can be intercalary (formed between hyphae) or terminal (on the ends of hyphae). They differ from hyphal swellings by having thick walls and are delimited from the mycelium by septa. The morphology of chlamydospores does not differ greatly between species and therefore these spores are of limited use in species identification. However, the presence (for example, *P. palmivora*) or absence (for example, *P. heveae*) of chlamydospores can aid species identification. Chlamydospores are generally produced readily in agar or water culture.

Sexual structures

Approximately half of the species of *Phytophthora* are homothallic. They will therefore produce oogonia, antheridia, and oospores in single culture. The remainder are heterothallic, with two mating types, A1 and A2. Heterothallic species produce gametangia (oogonia and antheridia) only in the presence of an isolate of the opposite mating type on the same plate. For species identification, it is important to determine if a culture is homothallic or heterothallic, and whether the antheridium is amphigynous (Figure 5.2) (around the oogonial stalk) or paragynous (next to the oogonial stalk).

A number of media are suitable for mating type tests, including cornmeal agar, carrot agar, Lima bean agar and kidney bean agar. Kidney bean extract contains anthocyanins that are incorporated into the oogonial wall, so they stain red, making them easy to see. Although the majority of species of *Phytophthora* produce oospores in culture, some species require specialised media containing additives such as sterols to induce oospore formation. In general it is best to start with carrot

agar, which works for most species. Place a 0.5 cm² plug of culture of the unknown isolate on one side of the Petri dish. Place an agar plug from the known A1 or A2 tester isolates on the other side of the dish. Incubate plates in the dark at the optimal temperature for the species being examined. Oospores should form at the junction of the two colonies (Figure 5.3) after 7–14 days if the isolates are of different mating types.



Figure 5.2 Amphigynous antheridia of *Phytophthora palmivora* oospore.

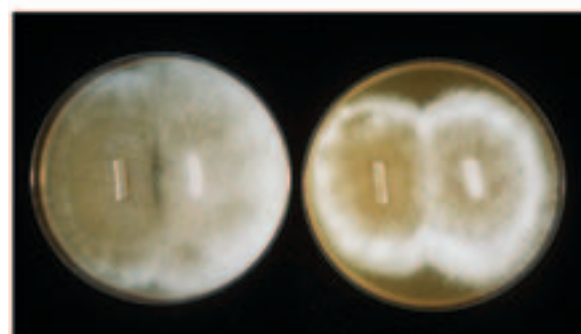


Figure 5.3 Oospore tester plate.

Differences between *Pythium* and *Phytophthora*

When isolating from soils one of the most common organisms one encounters is species of the genus *Pythium*. *Phytophthora* and *Pythium* belong to the Family Pythiaceae and hence are very closely related genera. Differences between the two include the following:

- Production of zoospores: in *Phytophthora*, the zoospores are produced within the sporangium, in *Pythium*, the zoospores develop within a vesicle produced by the sporangium. This is the most important distinguishing feature between *Pythium* and *Phytophthora*. Therefore, the second and third points below are provided for information only.

Table 5.2 Some common antibiotics: their activity, properties, preparation and alternatives.

Chemical	Activity	Target organisms	Preparation of stock solution	Stock (mg/ mL)	Range used ($\mu\text{g}/\text{mL}$)	Comments	Alternative
Ampicillin	Antibacterial	Gram +ve bacteria	Dissolve 1000 mg (1g) powder in 10 mL distilled water. Filter-sterilise	100	250-500		Penicillin G
Benomyl	Antifungal	Most fungi except Zygomycetes and Oomycetes	Benomyl is relatively insoluble in water. Add powder to media before autoclaving. Agitate media during pouring to ensure uniform distribution of suspension.	-	10-25	Does not suppress many undesirable fungi	
Hymexazol (HMI or Tachigaren)	Antifungal	Most <i>Pythium</i> spp.	Dissolve 500 mg powder in 10 mL distilled water. Filter sterilise	50	25-50	May inhibit some <i>Phytophthora</i> spp.	
Nystatin (Mycostatin)	Antifungal	Most fungi except the Peronosporales	Dissolve 500 mg powder in 5 mL distilled water. Filter-sterilise	100	10-100	Not active against some <i>Mortierella</i> spp. Not as active as Pimaricin; used at a higher concentration	Pimaricin
Penicillin G	Antibacterial	Gram +ve and Gram -ve cocci; Gram +ve bacilli	Dissolve 500 mg powder in 10 mL distilled water. Filter-sterilise	50	50-100	Not active against Gram -ve bacilli	Supplement with polymixin B at 50-100 mg/mL
Pentachloronitrobenzene (PCNB)	Antifungal	Narrow antifungal spectrum	PCNB is not soluble in water. However, it is heat stable so that the powder can be added to the media before autoclaving.	-	10-100	Does not suppress many undesirable fungi	
Pimaricin	Antifungal	Most fungi except the Pythiaceae	Does not dissolve in water. Mix 250 mg powder in 10 mL sterile distilled water. Do not filter-sterilise	25	2-100	Not active against some <i>Mortierella</i> spp. Dosage must be restricted to $\leq 10 \mu\text{g}/\text{mL}$	Nystatin
Polymixin B	Antibacterial	Gram -ve bacteria	Dissolve 500 mg powder in 10 mL distilled water. Filter-sterilise	50	20-50		
Rifampicin	Antibacterial	Gram +ve bacteria; Gram -ve bacteria to a lesser extent	Dissolve 100 mg powder in 10 mL ethanol (95%). Filter-sterilise	10	10		Penicillin G and polymixin B
Vancomycin	Antibacterial	Gram +ve bacteria; Gram -ve bacteria to a lesser extent	Dissolve 1g powder in 10 mL distilled water. Filter-sterilise	100	100-200	Very expensive	Penicillin G and polymixin B

Table 5.3 Baiting techniques for isolating *Phytophthora* from soil.

Species	Bait material	Procedure	Reference
<i>P. cinnamomi</i>	Apple or pear	Make holes in fruit. Fill with soil. Incubate covered with plastic bag at 15–27°C for 5–10 days.	Campbell (1949)
	Apple slices	Isolate from the edge of the rotted area around the hole. Suitable technique for many <i>Phytophthora</i> spp.	Gerretson-Cornell (1974)
	Avocado fruit	Immerse slices in 200 mL water to which 25 g soil has been added, for 4–10 days.	Zentmyer et al. (1960)
	Avocado seedlings	Embed fruit partially in flooded soil. Incubate at 20–27°C for 2–4 days.	Zentmyer (1980)
<i>P. citrophthora</i>	Avocado leaf pieces	Plant seedlings in wet soil. Incubate at 21–27°C for 2–3 days.	Pegg (1977)
	Apple, lemon or orange fruit	Float leaf pieces on water added to soil for 4 days.	Klotz and DeWolfe (1958)
	Lemon fruit	Insert soil or citrus tissue into fruit as per Campbell (1949) for <i>P. cinnamomi</i> . Alternatively, place lemon or orange on the surface of soil for 4 or more days	Tsao (1960)
	Lupin radicles	Immerse partially in 150 mL water to which 25 cc soil has been added. Incubate at 25°C for 6 days	Lee and Varghese (1974)
<i>P. heveae</i>	Apple fruit, eggplant fruit, cocoa pod	See Dance et al. (1975) under <i>P. cryptogea</i>	
<i>P. nicotianae</i>	Apple, lemon or orange fruit	No method is given but the baits could have soil inserted into them as per the apple method of Campbell (1949), or they could be embedded in partially flooded soil as per Zentmyer et al. (1960) for <i>P. cinnamomi</i> .	
	Citrus leaf pieces	See Klotz and DeWolfe (1958) under <i>P. citrophthora</i> .	Grimm and Alexander (1963)
	Cocoa pods	Float small leaf pieces on water 1–2 cm above 100 mL soil. Incubate at 22–28°C for 3–4 days.	Chee and Foong (1968)
	Lemon fruit	Insert soil or diseased rubber tissues into unripe green pods as per the apple method of Campbell (1949). Incubate at 26–30°C for 4–5 days.	Jenkins (1962)
<i>P. palmivora</i>	Tobacco leaves	See Tsao (1960) under <i>P. citrophthora</i> . Immerse the petiole end of leaf in water–soil mixture as per Tsao (1960) under <i>P. citrophthora</i> .	
	Apple fruit	As per the apple method of Campbell (1949).	Lee and Varghese (1974)
	Apple fruit, eggplant fruit, cocoa pod	No method is given but the baits could have soil inserted into them as per the apple method of Campbell (1949), or they could be embedded in partially flooded soil as per Zentmyer et al. (1960) for <i>P. cinnamomi</i> .	Holliday and Mowat (1963)
	Black pepper leaves	Immerse black pepper leaves or leaf discs partially in a water–soil mixture. Used for isolation from black pepper soils.	Orellana (1959)
	Cocoa pods	Place on or in soil. Or, inoculate pod surface with a small amount of soil suspension. Incubate in a plastic bag for 1–4 days. Used for isolation from cocoa soils.	Turner (1965)
	Cocoa pods	Insert soil beneath flaps of endocarp tissue of unripe cocoa pods. Used for isolation from cocoa soils	Newhook and Jackson (1977)
	Cocoa pods and tissues	See Chee and Foong (1968) under <i>P. nicotianae</i> .	Satyprasad and Ramaro (1980)
Taro roots	Place pod or pod tissue on soil, or in flooded soil. Soil can also be placed inside the pod. Cut roots into 2.5 cm lengths, autoclave. Incubate in moistened soil (50 g/Petri dish) at 15°C for 7 days.		

- Differences in the sporangia: the sporangia of *Phytophthora* are always terminal and usually ovoid or obpyriform in shape, whereas sporangia of *Pythium* may be globulose, lobate (many lobed), or filamentous and are frequently intercalary.
- Differences in the antheridia: in *Pythium*, the antheridia are paragynous and may be attached at any point on the oogonium, whereas in *Phytophthora*, the antheridium attaches only at the lower hemisphere of the oogonium. In addition, in some species of *Pythium*, many antheridia may be attached to a single oogonium.

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6

Major Crops Affected by *Phytophthora*



6.1 *Phytophthora* on Cocoa

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Abstract

Phytophthora pathogens are responsible for some of the most serious diseases of cocoa including phytophthora pod rot (PPR) or black pod, stem canker, leaf and seedling blight, chupon wilt and flower cushion infections. PPR causes 10–30% annual losses in production of cocoa beans globally, and much higher losses locally in particularly wet and humid conditions. Stem canker causes further losses and also tree deaths. Eight species of *Phytophthora* have been isolated from diseased cocoa, but most losses in production are caused by *Phytophthora palmivora*, *P. megakarya* and *P. citrophthora*, which cause similar diseases with slightly varying symptoms. Worldwide, *P. palmivora* is one of the most serious pathogens on cocoa, and in Southeast Asia this species accounts for almost all of the phytophthora diseases of cocoa. The most effective control measures are the introduction of resistant cocoa genotypes and farm management practices such as removal of infected pod husks, proper pruning of the canopy and judicious selection of shade species and associated crops.

Introduction

Among the numerous pathogens of cocoa (*Theobroma cacao* L.), species of *Phytophthora*, notably *Phytophthora palmivora* with a worldwide distribution and *P. megakarya*, which is restricted to West Africa, cause serious losses. Diseases of cocoa can be grouped into those that have spread with cocoa from its centre-of-origin in the Amazon region, and new-encounter diseases, which have transferred from other plants in regions to which cocoa has been introduced (Keane 1992). *Phytophthora* diseases probably fall into the 'new-encounter' group. The original hosts from which the various *Phytophthora* pathogens on cocoa transferred remain unknown. Since both *P. palmivora* and *P. megakarya* have a wide host range (Erwin and Ribeiro 1996; Opoku et al. 2002); it is likely that such transfers have occurred more than once. However, a study of the genetic diversity of isolates collected from different regions around the world suggests that at least some of the distribution of *P. palmivora* on cocoa outside its centre of origin

has been clonal, which suggests that it has spread with its host (Alex Appiah, pers. comm.).

Impacts of *Phytophthora* on Cocoa Production

The main regions of cocoa production are West Africa, Central and South America and Southeast Asia/Pacific, with more than half the world's cocoa being produced in West Africa (World Cocoa Foundation, <www.chocolateandcocoa.org/Supply/supplyindex.htm>). Southeast Asia, particularly Indonesia, is becoming an increasingly important centre of cocoa production. However, production in this region is affected by three main disease and pest problems: cocoa pod borer (*Conopomorpha cramerella*), vascular-streak dieback caused by *Oncobasidium theobromae* and phytophthora diseases caused by *P. palmivora* (Figure 6.1.1).

It is difficult to estimate yield losses due to phytophthora diseases since the same species may cause a number of diseases, and environmental conditions, particularly rainfall and humidity, can have a dramatic effect on disease incidence and severity (Thorold 1955; Tollenaar 1958). Most phytophthora-related losses can be attributed to phytophthora pod rot (PPR), followed by stem

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cankers. It is commonly estimated that 10–20% of the world's annual production is lost due to PPR, but estimates vary from average annual losses of 10% (Padwick 1956) up to 30% (Medeiros 1977; Opeke and Gorenz 1974), with much higher losses in particularly wet locations or wet years. In Western Samoa, losses of 60–80% due to PPR in wet years were reported by Keane (1992). Data collected at Keravat, Papua New Guinea, over an 18-year period indicate a mean annual loss of cocoa yield due to PPR of 17% and a range from 5–39% (Holderness 1992). In Mexico, losses of up to 80% due to PPR were reported by (Rocha 1965). Surveys in Java indicated that the percentage of pod rot ranged from 26 to 56% (Pawirosoemardjo and Purwantara 1992). If the impact of other phytophthora diseases such as stem canker were taken into consideration, these figures would be even higher. Stem canker contributes to production losses although these are difficult to assess, and can also cause tree deaths. A survey in Solomon Islands by Friend and Brown (1971) indicated tree losses to phytophthora canker averaged 3% annually over 5 years, with losses of trees approaching 40% in one locality.



Figure 6.1.1 Black pod in cocoa caused by *Phytophthora palmivora* in Indonesia.

Cocoa Agrosystems

Wild populations of *Theobroma cacao* in the Amazonian forest are shade adapted shrubby trees growing under the rainforest canopy. Over-storey shade trees used on cocoa farms include coconuts (particularly in Southeast Asia), legumes such as *Leucaena* and *Glyricidia*, and even rainforest trees left standing after partial clearing. The shady conditions produced by over-storey shade trees and the dense

foliage of cocoa itself provide favourable conditions for oomycete pathogens such as *Phytophthora* spp. Over-storey shading, unpruned cocoa canopies (self-shading) or high-density plantings can reduce the movement of air, leading to increasing humidity, highly favourable for *Phytophthora*. Conversely, removing shade trees completely may result in epidemics of *Colletotrichum*-related diseases and increase insect pest populations on cocoa (Smith 1981). To reduce pest and disease problems, a balance is needed that optimises both shade conditions and air movement within the cocoa canopy. Smith (1981) pointed out that, in Papua New Guinea, cocoa experiences fewer pest and fungal pathogen problems when grown under tall shade (e.g. coconut) than under low shade (e.g. *Leucaena*).

The choice and management of shade crops is important in integrated approaches to managing phytophthora diseases considering the fact that some shade trees (e.g. coconut) are also hosts of *Phytophthora* pathogens (Smith 1981; Opoku et al. 2002). The possibility that *P. palmivora* on coconut could infect cocoa trees growing on the same farm needs to be considered, although budrot is rare in the endemic tall palms of Southeast Asia. Judicious inter-planting with non-host plants (e.g. for wind breaks, insect breaks or alternative sources of income), or use of non-hosts as shade trees, could reduce transmission of *Phytophthora* infections. However, the economic value of the shade tree will also affect choice. In parts of Vietnam, where the cocoa industry is relatively new, durian trees, which are affected severely by *P. palmivora*, are the shade species of choice on cocoa farms because of the high financial returns from durian fruit (David Guest, pers. comm.). An important question in these areas will be whether *P. palmivora* can cross infect between the two tree crops and give rise to increased disease problems on both host plants. Disease management in intercrops and mixed plantings has to include all components, although mixed plantings are less vulnerable to explosive epidemics seen in monocultures.

Phytophthora Pathogens of Cocoa

Phytophthora pathogens thrive on all parts of the cocoa plant from the seedling to mature stages, causing a number of diseases. To date, eight species of *Phytophthora* have been isolated from cocoa: *P. palmivora* (Butler) Butler, *P. megakarya* (Brasier and Griffin), *P. capsici* (Leonian emend.) (= *tropicalis*), *P. katsurae* (Ko and Chang), *P. citrophthora* (R.E. Smith and E.H. Smith), *P. arecae* (Coleman) Pethybridge, *P. nicotianae* (van Breda de Haan) and *P. megasperma* (Dreschler) (Erwin and Ribeiro 1996; Iwaro et al. 1997; Appiah et al. 2003). Throughout the world

most damage is caused by *P. palmivora* and, in particular localities, by *P. megakarya* and *P. citrophthora* (Brasier and Griffin 1979; Brasier et al. 1981; Kellam and Zentmyer 1981). These three pathogens cause similar diseases including PPR and stem canker, although symptoms and pathology may vary slightly (Lass 1985). For example, in West Africa both *P. palmivora* and *P. megakarya* infect cocoa pods, causing pod rot or black pod and both these species also cause stem cankers.

Following Turner's identification of distinct strains of '*P. palmivora*' isolated from West African cocoa (Turner 1960), Brasier and Griffin (1979) designated three morphological forms, MF-1, MF-3 and MF4 as separate species. Only MF-1 was clearly *P. palmivora*. MF-4 was identified as *P. capsici* or a similar species and MF-3 as a new species, *P. megakarya*. MF-4 has recently been described as a separate species, *P. tropicalis* (Aragaki and Uchida 2001). Possibly other taxa will be found in the *P. capsici*-*P. tropicalis* complex (Appiah et al. 2003). MF-2 (Waterhouse 1974b) was not accepted as a valid taxon by Brasier and Griffin (1979).

Chowdappa and Mohanan (1996) reported that PPR in India was associated with *P. citrophthora*. This pathogen has been reported to occur on cocoa in Brazil (Campelo and Luz 1981; Kellam and Zentmyer 1981), in Cameroon (Lass 1985) and in Indonesia (Appiah et al. 2003). In Brazil, *P. capsici* is often isolated from PPR-affected pods (pod lesions) along with *P. palmivora* (Campelo and Luz 1981), although it is likely that the main causal pathogen is *P. palmivora*. *P. capsici* has been reported on cocoa in Kerala, India (Chowdappa and Mohanan 1996). *P. megasperma* was found on cocoa in Venezuela (Zadoks 1997) and *P. katsurae* on cocoa in Sri Lanka (Liyanage and Wheeler 1989). *P. palmivora* is the main species attacking cocoa throughout Southeast Asia where, under conditions favourable it is able to infect the pods at all stages of development (causing pod rot and cherelle wilt), the flowers and flower cushions, the main trunk (causing cankers which sometimes lead to death of the tree), the chupons (causing chupon wilt), the young growing twigs and young leaves of mature trees sometimes leading to repeated defoliation, dieback and death of the tree, the petiole and lamina of old leaves (causing leaf blight), and the young seedlings (causing seedling blight) (Gregory 1974; Lass 1985).

Few studies have been done to compare the pathogenicity of different species or different isolates of *Phytophthora*. In one study, Kellam and Zentmyer (1981) transplanted germinated cocoa seeds into soil artificially infested with

chlamydospores or oospores of *P. palmivora*, *P. citrophthora* or *P. capsici*. After 8 weeks, they found that *P. capsici* had not caused any seedling mortality, while infection with *P. palmivora* and *P. citrophthora* resulted in mortality rates of 67% and 53%, respectively. In Brazil, Campelo et al. (1982) reported that, on healthy, detached pods, *P. citrophthora* was more pathogenic than both *P. palmivora* and *P. capsici* (see Lass 1985). Liyanage and Wheeler (1989) found that, compared to *P. palmivora*, *P. katsurae* is only mildly pathogenic. Five days after inoculation of healthy, detached pods, *P. palmivora* had produced over 10-fold larger lesions than had *P. katsurae*.

Disease symptoms

Phytophthora pod rot begins on the surface of the pod. Infection starts as a discoloured spot, then develops into a brown or black lesion with a well-marked boundary, and spreads over the entire pod within about 2 weeks. On older pods, infections mostly start at either the tip or the stem end of the pods. Equatorial infections are usually associated with damage to the pod surface or wounds. The rot involves the whole of the fleshy tissue of the husk as well as the pulp and seeds (Figure 6.1.2). Infection of pods approaching ripeness when the seeds are no longer in close contact with the husk may not lead to infection of the seeds, which therefore can be salvaged and fermented. The pathogen appears on the surface of the pod as a whitish down on which masses of sporangia are produced. The pod ultimately blackens and shrivels, and is colonised by secondary fungi. PPR is a firm rot that can be distinguished from pod rot caused by *Botryodiplodia theobromae*, which causes loss of firmness in the pod wall and relative dryness of the diseased tissue (Thrower 1960a), and from infections by *Colletotrichum* which result in dark, often sunken lesions. Cherelle wilt (Figure 6.1.3) may be caused by *P. palmivora* but this needs to be distinguished from physiological wilt which may be related to stress associated with excessive fruit set (Thrower 1960b).

Stem canker is characterised by development of brown necrotic bark around the trunk. When the surface of the bark is scraped off, the affected tissues become watery to gummy and of a dull brownish-grey colour that often assumes a claret tone on exposure (Figure 6.1.4). The necrosis does not extend into the wood beyond the cambium layer. When the canker enlarges, it may encircle the trunk, causing 'sudden death' of the tree. In Papua New Guinea, cankers were found to be associated with channels made by larvae of the insect pests, *Pantorhytes* and *Glenea* (Prior and Sitapai 1980). Additionally,

contaminated pruning implements, diseased pod peduncles and diseased pods in contact with the bark are sources of inoculum (Vernon 1971; Brown and Friend 1973). Flower cushion cankers result from contaminated harvesting knives, or by visits from flying beetle vectors (Konam and Guest 2004).



Figure 6.1.2 Black pod rot on the inside of infected pods.

The pathogen naturally attacks and kills unhardened (flush) leaves and young green stem tissue. It also infects mature leaves, even though this is not normally regarded as being serious (Manco 1966). Infection of flush leaves and stems can lead to death of the growing point or of the whole plants in the case of seedlings, and can cause bark cankers when the pathogen spreads down a chupon (chupon wilt). Cocoa seedlings grow very rapidly in the first few months and produce young leaves that are highly susceptible to pathogen attack.



Figure 6.1.3 Cherrille wilt in cocoa

Disease cycle

On cocoa farms, *Phytophthora* is dispersed by rainsplash (from infections on the plant, often as

sporangia, and from the soil), and by vectors such as ants and flying insects (Dade 1927, 1928; Evans 1971, 1973a,b; Gregory et al. 1984; Konam 1999; Konam and Guest 2004). The most important infective propagules of *Phytophthora* are motile zoospores. Rainsplash probably disperses sporangia (*Phytophthora* spp. on cocoa have deciduous sporangia) followed by release of zoospores. Encysted (dormant) zoospores, chlamydospores and hyphae might be other forms of inoculum (Turner 1965; Gregory et al. 1984). Both *P. palmivora* and *P. megakarya* can survive for up to 4 months in cocoa roots and soil, as was shown by Opoku and Wheeler (1998) (Konam and Guest 2002).

For *P. palmivora* in the Southeast Asia-Pacific region, flower cushions are likely to be particularly important reservoirs of infection (Brown 1973). Additionally, infected plant parts and cocoa pods left on the ground or in the canopy after harvest (especially as there is a tendency not to harvest black pods) provide a large proportion of inoculum for *Phytophthora* pathogens generally (Ward and Griffin 1981; Purwantara and Pawirosoemardjo 1990; Konam 1999).



Figure 6.1.4 Stem canker in cocoa tree

In Nigeria, where the predominant *Phytophthora* pathogen is *P. megakarya*, a long-term research study on PPR demonstrated that rainsplash from or contact with infected pods accounted for more than 71% of pod losses (Gregory et al. 1984). Other sources of infection included soil (5%), ant tents (5.8%) and pod-damage due to insects and rodents (4.9%) with 10.9% attributed to 'no obvious' sources. Rather than disease spreading from a few initiator pods, it appeared it spread from numerous 'initiator' pods with sources for these initial infections being partly derived from the soil and ant tents, but also largely (40%) from 'no obvious sources' (Griffin et al. 1981; Gregory et al. 1984). Observations on infection sources of Amazonian, Amelonado and Trinitario cocoa types in Java for three years (1990–1992)

showed similar results (Purwantara 2003). Contact or splash from infected pods accounted for about 35% of infection. On average, living vector activity accounted for about 14%, whereas infection from soil and cankers was only 3% and 7%, respectively. Almost 40% of sources of infection were not identified (no obvious source) (Table 6.1.1). This high percentage could be due to the activity of living vectors such as squirrels and rats, which carry spores that are disseminated onto healthy pods. Almost 12% of infection was associated with rodent damage.

In addition to the possibility that inoculum is carried upwards by convection of aerosol-sized water droplets as well as larger rain-splashed drops (Gregory et al. 1984), tent-building ants are likely to be important agents of vertical spread (Evans 1971, 1973a,b; Newhook and Jackson 1977; Gregory et al. 1984; McGregor and Moxon 1985; Konam 1999). In Papua New Guinea, Konam (1999) established that tent building and/or path building ants were strongly associated with PPR incidence. When Konam dislodged ants and ant tents from cocoa trees and then prevented access of ants from the soil by applying grease near the base of the trees, the incidence of PPR was significantly lower than in untreated trees, and the treatment also led to significantly increased yields. These results were obtained even when infected pod husks were scattered under the trees, suggesting that ants, rather than flying insects, provide most of the inoculum that infects healthy pods. However, it is apparent that flying insects also play an important role in inoculum dispersal (Konam and Guest 2004).

Potential agents of horizontal spread of *Phytophthora* are wind-dispersed spores or water droplets and flying insects and other fauna. Wind appears not to be an important factor in horizontal spread of *Phytophthora* (Evans 1973a; Wharton 1955). However, in West Africa, Thorold (1954, 1955)

trapped zoospores above infected pods, indicating some spores were wind dispersed (Waterhouse 1974a). Konam (1999) established that in Papua New Guinea two types of flying beetle, a scolytid and a nitidulid, preferentially visited and bored holes in infected pods. The beetle frass contained viable spores. He concluded that the beetles' frass provided a new source of inoculum that could be dispersed by water and perhaps wind (the dust-like frass could be blown around).

Intra-specific Diversity of *Phytophthora* Species

Both mating types of *P. palmivora*, A1 (once called the 'rubber' type) and A2 (the 'cacao' type), are found on cocoa with the A2 mating type predominating (Turner 1961; Zentmyer 1974). Of 70 *P. palmivora* isolates collected from around the world by Appiah et al. (2003) only 16 were of the A1 mating type. In contrast, 19 of 29 *P. palmivora* isolates collected from non-cocoa hosts for the same study were predominantly of the A1 mating type. Oospores have never been found in the field on cocoa, although they are obtained in culture when A1 and A2 types are plated together (Tarjot 1974).

Turner (1961) found that isolates of *P. palmivora* collected from cocoa around the world were remarkably uniform morphologically, consistent with sexual isolation (Zadoks 1997). Brasier and Griffin (1979) and Appiah et al. (2003) also found that the morphology of international collections of *P. palmivora* was relatively uniform. Furthermore, molecular studies indicated that *P. palmivora* isolates collected from different regions around the world (including Central America, West Africa, Southeast Asia, Taiwan and Papua New Guinea) have a greater genetic uniformity than *P. megakarya* isolates collected from different regions of Africa (A.A. Appiah et al., unpublished data).

Table 6.1.1 Percentage of incidence of pod infection on three cocoa types in Java during 1990–1992. Source: Purwantara (2003).

Likely source of infection	Incidence of pod infection (%)			Mean incidence of infection (%)
	Amazonian	Amelonado	Trinitario	
Contact/splash from another pod	39.6	33.3	33.5	35.5
Soil and litter	2.8	5.7	1.5	3.3
Cushion and canker	8.4	7.2	6.2	7.3
Rodent damage	7.9	12.0	14.9	11.6
Ant tent	0.3	0.1	0.2	0.2
Harvest damage	2.6	1.8	1.5	2.0
Insect damage	1.8	3.2	1.7	2.2
No obvious source	36.6	36.7	40.5	37.9

Important questions requiring further study are the host-specificity of different isolates of *P. palmivora*. A case where direct inoculation demonstrates that a rubber isolate can infect cocoa, for example, might be more complex in a field situation where a variable *P. palmivora* population may be present. Thus, any *P. palmivora* population may contain a range of strains, only some of which are pathogenic and only some of these being able to infect more than one host, the others being host specific.

Host Resistance

Despite its obvious importance in disease control, the study of resistance to *Phytophthora* in cocoa has been neglected. There has been much confusion about methods for studying and measuring resistance. For example, resistance to stem canker may not be linked to resistance to PPR. The Forastero clone, Sca-6, is resistant to PPR (Okey et al. 1995) but susceptible to canker (Okey et al. 1996), and in Papua New Guinea, the KA2-101 clone is susceptible to PPR (McGregor 1981) but is less affected by canker (Prior and Sitapai 1980).

Resistance of particular cocoa clones observed in one country may not be evident in another, presumably due to varying environmental conditions or variations in the pathogenicity of different regional populations of *Phytophthora* (e.g. Lawrence 1978; Saul 1993). Resistance found in laboratory and glasshouse studies is not always evident in the field. The interaction of different species, and possibly strains, of *Phytophthora* is another factor to consider, although Zadoks (1997) considers that there is little evidence to contradict the hypothesis that host resistance to PPR is effective against different *Phytophthora* pathogens. When testing 10 cocoa clones for resistance to *P. palmivora* and *P. capsici*, Iwaro et al. (1998) obtained a similar ranking order, although *P. palmivora* was the more aggressive species. Another problem is that resistance tests on detached plant parts might not correlate with results from attached plant parts, although Iwaro et al. (1997) found that results from resistance tests on leaves and pods were similar whether they were detached or attached.

Despite all the above-mentioned difficulties, in cocoa-growing countries, there are consistent differences in the incidence of pod rot and canker on different varieties. In Indonesia, phytophthora diseases are generally most severe in Criollo type varieties. At the beginning of the last century, canker was very serious in Java, leading to the eradication of a very susceptible Criollo-type (Van Hall 1912, 1914). However, canker is no longer a menace in this

area since Criollo has been replaced by relatively more resistant Forastero types or Criollo-Forastero hybrids (Tollenaar 1958).

Amelonado varieties are also susceptible to canker. The cocoa genotypes currently widely planted in Malaysia, Indonesia and Papua New Guinea are mostly hybrids between Upper Amazon and Trinitario types, with Amelonado types in Sabah and Sulawesi, which are susceptible to pod rot and canker to different degrees. In Indonesia smallholder cocoa plantations are genetically diverse, and hybridisation occurs between out-crossing genotypes, making local selections a promising source of resistance.

Some sources of resistance to *Phytophthora* are found in varieties from Upper Amazon, Costa Rica (crosses between Trinitarios and an Amazon-type local genotype), Bahia (Catongo and related clones) and Ecuador (e.g. the clones Sca-6, Sca-12) (Soria 1974). Van der Vossen (1997) lists some cocoa clones with demonstrated resistance to *P. palmivora*, including P7, PA-150, EET-50, IMC-47, Sca-7, Sca-6, Sca-12 and K82. In Malaysia, PBC-123 and BR-25 are recommended for PPR resistance. In Papua New Guinea, long-term studies have shown differences between clones in their resistance to *Phytophthora* (Saul 1993). For example, in a particular year, K82 has been consistently ranked with a lower disease incidence compared to other clones over a number of years (Figure 6.1.5).

PPR resistance is mostly partial, involving reduced incidence of pod infection and reduced rates of expansion of lesions on infected pods (Saul 1993). However, A.J. McGregor (unpublished data) recorded varying responses in lesion development. Some lesions were small black spots or even barely discernible, consistent with restricted expansion due to cell death (Saul 1993). Phillips-Mora and Galindo (1989) also described some reactions of pods that were similar to the sudden collapse of tissues associated with hypersensitive necrosis. However, resistance to PPR controlled by a single gene with a strong effect has not been demonstrated. Resistance to *Phytophthora* in certain clones (e.g. Sca-6, K82, RJ-2) appears to be durable on the evidence that field tests have been conducted over a long period of time and no erosion of resistance has been observed (Figure 6.1.5).

Mechanisms of Resistance

Mechanisms of resistance in cocoa to *Phytophthora* pathogens are poorly understood. Iwaro et al. (1997) identified two aspects of resistance to *Phytophthora* operating at the penetration and post-penetration

stages of infection, with the poor correlation between the two suggesting that they are independent. The PPR-resistant Sca-12 clone had a high number of small lesions on pods (indicating a post-penetration rather than penetration mechanism of resistance), but it had few lesions on leaves (indicating resistance at the penetration stage in leaves). The authors therefore concluded that leaf tests for resistance could not be used to indicate resistance in pods. However, others have found good correlation between expressions of resistance in leaves (or leaf disks) and pods (Van der Vossen 1997).

Okey et al. (1995) compared the response of six genotypes of 3-month-old cocoa plants inoculated with *P. palmivora* into wounds in the stem. They found that larger lesions were obtained in genotypes that produced lower quantities of lignin at the wound sites, while poor correlation was found between lesion size and other wound healing components (suberin and callose). In a further study with 6-month-old cocoa, Okey et al. (1996) found that lower resistance to canker was associated with relatively low levels of bark hardness and relatively high levels of moisture in the bark.

Control of Diseases Caused by *Phytophthora*

Farm management practices

Various cultural management practices employed on cocoa farms can effectively control phytophthora diseases, particularly in conjunction with a degree of host resistance (Muller 1974; Toxopeus 1974). Disease is prevalent in wet areas. Humidity levels of nearly 100% during the night result in condensation of free water, which is essential for infection. Disease

incidence is increased by poor drainage of the plantation, and high humidity due to a heavy canopy and low branching of the trees. Pruning of cocoa and removal of low branches, combined with a reduction of shade to the minimum required for good growth of the cocoa, can contribute substantially to the control of phytophthora diseases. Not only does pruning allow increased air circulation and more rapid drying of the pod surfaces, but it facilitates complete harvesting of pods (including infected pods) and application of fungicide if required.

Cultural practices involving sanitation contribute substantially to control of phytophthora diseases in cocoa, although experimental studies are needed to quantify this. Such practices include regular complete harvesting of both healthy ripe pods and any infected pods, including pod mummies, which can remain sources of infection for long periods, and burying of infected pods and pod husks. Addition of manure (e.g. green vegetable matter plus chicken manure) can be used to hasten decomposition of pod remains and encourage the release of ammonia and stimulation of saprophytic microbes that will kill *Phytophthora* (Konam 1999; Konam and Guest 2002). Occasional application of a protective fungicide (e.g. in the dry season) or trunk injection of phosphonate could be used to kill surviving inoculum sources (in flower cushions, pod mummies and rough bark).

Chemical control

Copper fungicides have been used since the early 1900s to control pod rot (Tollenaar 1958). Cuprous oxide has consistently been shown to give good control of the disease (Newhall 1967). Metalaxyl became available in the late 1970s, and was found to be effective in controlling the disease (McGregor

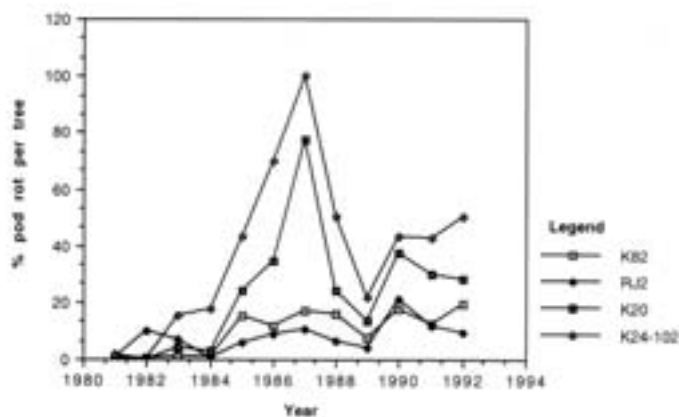


Figure 6.1.5 Percentage pods per tree infected by phytophthora pod rot in four Keravat clones, for the period July 1981 to December 1992 (from Saul 1993)

1982, 1984). The timing of application may be important (Mabbett 1986). However, these sprays provide only limited protection, particularly during the wet season when heavy rains are likely to wash away chemical treatments. Also, development of resistance to metalaxyl is likely since such resistance developed in *P. infestans* on potato crops (Erwin and Ribeiro 1996). Even if fungicides are effective, factors such as potential yield of the cocoa tree and cocoa prices have to be considered in determining the profitability of fungicide spraying (Fagan 1984; McGregor 1983).

Work with mature cocoa in Papua New Guinea demonstrated that PPR and stem canker could be controlled effectively by potassium phosphonate applied by injection (Anderson and Guest 1990; Guest and Grant 1991; Guest et al. 1994). Yields were almost doubled with twice-yearly trunk injections of 10% phosphonate solutions (Anderson and Guest 1990; Guest et al. 1994). Phosphonate is a simple inorganic compound that apparently operates in conjunction with physiological factors in the plants. Since it appears to be more toxic to the pathogen in planta than in vitro (Guest and Grant 1991). It specifically controls oomycete pathogens and is also more economic for the farmer than other treatments (Guest et al. 1994). Moreover, it avoids the problem of removal of surface treatments by rain, and involves very simple equipment (hand-drill and spring-loaded syringes). Uptake of this method has been slow; – Indonesian growers, for example, have been reluctant to adopt this control technique because of the wounding that results from multiple and regular injections (Yohannes Junianto, pers. comm.).

Biocontrol and Natural Plant Extracts

Odigie and Ikotun (1982) showed that *Botryodiplodia theobromae*, *Gliocladium roseum*, *Penicillium* spp., *Bacillus cereus* and *B. subtilis* inhibit the growth of *Phytophthora palmivora* in vitro and in vivo.

Plant extracts are another possible 'biological' treatment and testing such extracts against various pathogens is very active in some tropical countries. Awauh (1994) identified plant extracts that suppress PPR lesion development but their effectiveness is too short-lived (only 3 hours) to be useful for control purposes. Chapter 7.5 describes the development of microbial biocontrol agents for the control of black pod.

Selecting and breeding for resistance

Since cocoa genotypes are highly variable, and resistance to *Phytophthora* pathogens has been

evident in the field, there is a great deal of potential for deployment of more resistant genotypes (Toxopeus 1974; Zadoks 1997).

The resistance observed to date has been partial, additively inherited and apparently durable, and so is likely to be of long-term benefit to farmers. Quantitative trait loci (QTLs) in cocoa linked to *Phytophthora* resistance have been identified (Flament et al. 2001), providing a promising approach to improving predictability of resistance and thereby speeding up breeding programs (Van der Vossen 1997; Zadoks 1997).

Resistance to *Phytophthora* has been identified in some cocoa clones (see Host resistance), but these clones may not be suitable for propagation. For example, Sca-6 and Sca-12 are quite resistant to *Phytophthora*, but have a small bean size. To date, there has been little attempt to incorporate genotypes with known resistance to PPR (like Sca-6, PA-7, K82) into cocoa breeding programs. Such clones could be crossed with agronomically desirable clones to produce hybrids from which a wider range of genotypes with resistance could be selected on farms.

Rapid screening methods involving inoculation of pods, leaves or leaf disks may save considerable time and labour, since screening for resistance to phytophthora diseases in the cocoa field can take years (Blaha 1974; Lawrence 1978; Zadoks 1997). Good correlation may be found between rapid screening methods, such as leaf disc tests, and field tests (Nyasse 1997; Efron and Blaha 2000). It is important that rapid screening be supplemented by confirmation of resistance in the field. Saul (1993) developed an inoculation method in the field by transferring inoculum onto a pod by tape (the 'band-aid' method). This allows rapid assessments for resistance (Figure 6.1.6).

In Indonesia, trees relatively free of PPR have been observed next to heavily infected trees (Arief Iswanto, Indonesian Coffee and Cocoa Research Institute, Jember, pers. comm.). In Indonesia and Papua New Guinea, farmers recognise trees with superior yield of healthy pods; such trees are likely to have a degree of resistance to phytophthora diseases. These trees can be propagated clonally for experimental testing of their performance. Budwood can be side-grafted onto existing trees on a farm, allowing on-farm selection for PPR resistance. For example, farmers and extension officers could select budwood from potentially resistant cocoa genotypes and side-graft these onto susceptible genotypes or any rootstock that is available. The mother tree can eventually be pruned back to allow

the side-grafted resistant genotype to replace the original tree. This approach has been initiated by an ACIAR project (PHT/2000/102) based in Sulawesi, Indonesia. It is very suitable for the smallholder farmer and local extension services, since improvement of cocoa stock can be achieved without the need for inputs of expensive technologies or expertise. Field experiments established by that particular ACIAR project will test the efficacy of this approach as well as shed light on some unknown aspects such as the effect of susceptible rootstock on the grafted genotypes selected for their resistance.



Figure 6.1.6 Artificial inoculation of pods using the 'band-aid' method: one drop (0.1 mL) of a suspension containing zoospores, sporangia or a mixture of both is placed on the central absorbent pad of a band aid which is then pressed onto the pod surface. Band-aids or modified tape moistened with distilled water can also be used to hold in place discs of agar containing mycelium or epicarp plugs of infected tissue (Saul 1993).

Conclusion and Recommendations for Future Research

Developing host resistance to *Phytophthora* pathogens is the most pressing need in attempting to achieve control of phytophthora diseases in cocoa. A wide genetic base is fundamentally important for selecting and breeding for disease resistance. Therefore, the promotion of sound conservation strategies for a wide range of cocoa germplasm should be an integral part of dealing with phytophthora diseases. In addition to establishing collections of germplasm, the maintenance of on-

farm genetic variability in cocoa, which will enable local and environmentally relevant programs of selection and breeding, needs to be given serious consideration.

The importance of this is illustrated by the lack of success in selecting for disease resistance for swollen shoot virus in West African cocoa, which is largely derived from a few introductions and is genetically uniform Amelonado (Keane 1992).

In contrast, in Indonesia and Papua New Guinea, original introductions of Trinitario cocoa resulted in a high degree of genetic variability following propagation of seedlings. Since the 1960s, introductions of Amelonado and Upper Amazon material, hybridisation between all types of cocoa in mixed plantings and propagation of hybrids, have greatly increased the genetic diversity of cocoa on farms. This has allowed selection and breeding for disease resistance based on observations of resistance in the field, an approach that has been very successful in controlling vascular-streak dieback caused by *Oncobasidium theobromae* throughout the region (Keane 1992) but has yet to be fully exploited to control PPR and stem canker.

In addition to improving host resistance to *Phytophthora* pathogens, integrated disease management strategies are needed that take account of the disease cycles of *Phytophthora* pathogens of cocoa and the wider agrosystem within which cocoa is grown (Smith 1981). Information on the genetic diversity of *Phytophthora*, host-pathogen compatibility and variations in pathogenicity among *Phytophthora* populations between different cocoa-growing regions will be useful for adopting management schemes for cocoa agrosystems. Basic measures such as choosing appropriate shade species (preferably non-hosts of *Phytophthora*), pruning the canopy to improve air circulation and light penetration (which could kill zoospores), soil surface treatments such as mulching and manuring that suppress populations of *Phytophthora* in the soil, regular complete harvesting of both healthy and infected pods to reduce carryover of inoculum sources on the trees, burial of pod cases and infected pods to reduce inoculum at the soil surface, and the use of clean farm implements can all go a long way towards successful management of phytophthora diseases (See Chapter 8.5).

Combining cultural management methods with improved resistance could act to reduce disease synergistically, not just additively. Thus, cultural methods of phytophthora disease control might be quite ineffective on very susceptible cocoa, but show

dramatic results as soon as partially resistant clones are used. As is the case with most *Phytophthora* pathogens in tropical regions, no one control measure can hope to contain phytophthora diseases on cocoa, rather the diseases need to be managed using an integrated approach that aims to minimise losses.

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6.2 Phytophthora Diseases of Coconut in the Philippines

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Abstract

Coconut is an economically important crop for the Philippines and is the number one export product. Although *Phytophthora palmivora* was known to cause bud rot, and fruit and immature nut fall in the Philippines, the disease losses were relatively low. This changed dramatically after the introduction of highly susceptible MAWA hybrids, which are a cross between Malaya Yellow Dwarf and West African Tall. This chapter provides an overview of the impact of the introduction of this material on coconut production in the Philippines.

Introduction

The coconut (*Cocos nucifera* L.) is a monoecious plant and member the palm family, and is a major earner of foreign exchange for the Philippine economy. The crop provides income directly or indirectly to about one third of the country's population. The coconut industry is considered to be a major pillar of the Philippine economy, supporting 3.4 million farm families directly dependent on coconuts for their livelihood, and a further 24 million individuals who are indirectly dependent on the industry, such as traders, exporters, processors, and their employees. Three hundred million coconut palms spread over 4.09 million ha dominate the landscape of 65 of the 78 provinces in the country. Among the 15 administrative regions of the Philippines, Southern Luzon had the largest area under cultivation (19%) followed by Bicol (16%), Eastern Visayas (15%), and Southern Mindanao (12%). Coconut remains the number one agricultural export product, having generated aggregate foreign exchange earnings of USD768.5m during 1991–2000.

The Philippines was the number one coconut producer in the world during 1976–1986. However, the average productivity has declined in the past decade (1991–2000) with an average production of 669 kg/ha. It lags behind India, which produces, on

average, 732 kg/ha, and Indonesia with an average production of 1041 kg/ha. This lower productivity can be attributed to a number of factors, such as slow adoption of recommended cultural management, an increasing number of senile trees, and damage brought about by pest and disease outbreaks. Bud rot and fruit rot were major causes of the large loss of coconut trees and the significant decrease in production.

Bud rot, an apical meristem decay (Reinking 1923) and fruit rot or immature nutfall (Teodoro 1925) are two destructive diseases known to be caused by *Phytophthora palmivora* in coconuts. As well as in the Philippines (Concibido 1990), these diseases were reported to have caused significant coconut yield losses in the Ivory Coast (Quillec et al. 1984) and Indonesia (Bennett, Roboth et al. 1986), in areas planted with the MAWA hybrid. This is a cross between the Malayan Yellow Dwarf and West African Tall varieties, both of which are known to be susceptible to phytophthora.

In the Philippines, bud rot was the first reported disease of coconut and was observed by Reinking in 1919 causing the death of local plantings. The disease never reached epidemic proportions and was known to be prevalent only in the highlands, where the climatic conditions favour disease development. It was only in 1989 that the Philippine Coconut Authority (PCA) became alarmed by the reported outbreak of bud rot that caused the death of over 3000 MAWA hybrid trees in large coconut

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plantations. These included the 600 ha Ayala Agricultural Development Corporation (AADC) and the 700 ha coconut plots of the Cocoa Investors Inc. (CII) in the southern part of the island of Mindanao in the Philippines. Likewise, at pilot hybrid farms (PHFs) bud rot of the MAWA hybrids was prevalent and regularly monitored by the PCA.

Immature nut fall did not gain attention after Teodoro (1925) gave a detailed description of the disease with the observation that it did not cause significant losses in production. No further reports of the disease were made until 1986, when immature nutfall was reported to be causing significant nut losses in the germplasm collection plots of PCA and in the MAWA PHFs.

It was speculated that the plantings of the MAWA hybrids were one of the main factors that escalated disease incidence in the country, due to its susceptibility to phytophthora infection. It was believed that the genetic uniformity of the nationwide large-scale plantings of MAWA was the major factor that led to the development of disease epidemics between 1989 and 1992. The death of over 1000 palms in the PHFs indicated the potential threat of phytophthora diseases to the coconut industry in the Philippines.

The Disease

Coconut bud rot has been known to be in the Philippines since 1919 when it was reported on the foot slopes of Mt Banahaw on Luzon Island. The disease was considered to be the first serious infectious disease of coconut that causes death of palms. Early epidemics were reported in the highlands of Quezon and Laguna, and sporadic diseased trees were identified in Bukidnon in 1976 (PCA, Crop Protection Guidebook, 1977).

Early studies of the nature and aetiology of bud rot were undertaken by Reinking in 1919. They are considered as the pioneering studies in plant disease which mark the start of plant pathology in the Philippines. Unfortunately, after this initial work, no further studies were conducted due to the sporadic and infrequent incidence of the disease. Information available about the disease and its host-pathogen interaction before the introduction of the MAWA hybrid is therefore rather limited.

The PCA took serious action against the disease only when it was reported to be widespread in the 50,000 ha plantations of MAWA hybrids located on the Ivory Coast. A large-scale replanting program based on the high-yielding MAWA hybrid was under way at the time, and the death of over 1000 palms to bud

rot highlighted the potential threat to the coconut industry if the replanting program were to continue. A few cases of the disease were reported on local cultivars but were mostly confined to the highlands, where the climate is humid with a long wet season that is conducive to disease development.

After Teodoro's detailed description of immature nutfall in 1925 (Teodoro 1925), no further cases of the disease were reported. It was only in 1986 that phytophthora-induced nut fall was reported to be prevalent in the germplasm collection plots of the Zamboanga Research Center (PCA-ZRC). It was first observed in the nuts of the Red Cameron Dwarf (RCD) plantings and later in the Malayan Red Dwarf (MRD) and Malayan Yellow Dwarf (MYD) collection plots. Rillo and Paloma (1988) noted that red and yellow pigmented nuts are more susceptible to nutfall than green ones, based on 5-year observations of the disease incidence amongst the various populations planted in the PCA-ARC. Nuts with symptoms of fruit rot or immature nutfall were also found in some PHFs where MAWA had been planted, particularly on Mindanao. To date, there has been no report of fruit rot incidence in local plantings, or in the PCA-ZRC and Davao Research Centers (PCA-DRC) planted with local hybrids.

The Pathogen (*Phytophthora palmivora* Butl.)

Four species of *Phytophthora*, *P. palmivora*, *P. arecae*, *P. katsuriae*, *P. nicotianae*, have been implicated as the causal organisms of the bud rot and fruit rot diseases of coconut (Quillec et al. 1984). Recent studies conducted to elucidate the pathogenic nature of these four species have produced inconclusive results.

Isolation of the oomycete organisms of the genus *Phytophthora* proved to be difficult in the initial studies. Isolations from plants in the advanced stages of bud rot were generally unsuccessful, since infected tissues are prone to contamination with other fungi and bacteria. Only in the early stages of disease development can the pathogen be found at the edges of infected areas or lesions, and sometimes in the centre as mycelium (Quillec et al. 1984). Based on an initial morphological identification of *Phytophthora* isolated from sporulating infected nuts, *P. palmivora* was declared to be involved in immature nutfall. However, it was later reported that several species of *Phytophthora* can attack coconut buds and nuts, and so taxonomic studies were conducted to identify the pathogen based on morphological and molecular characteristics (Chee 1969).

In the Philippines, *Phytophthora* samples isolated from infected nuts and bud are usually identified as *P. palmivora* (Reinking 1923). This pathogen produces a 'dry' rot before the development of rotting symptoms that are associated with other organisms such as *Fusarium* and *Erwinia* species (Joseph and Radha 1975). It was observed that, while *Phytophthora* is the primary causal agent of the disease, rotting of the bud and subsequent maceration of tissues and foul odour emission are triggered by bacterial infection. At this stage, it is no longer possible to isolate the primary cause of the disease from bud tissues.

It was noted that, in the case of fruit rot, *Phytophthora* species could be isolated from the perianth area and sometimes from the peduncle of the inflorescence. Water-soaked lesions were observed on the epidermal portion of the nut, which becomes brownish at advanced disease stages, and premature senescence results in the nut falling from the bunch. It was claimed that the organism penetrates the soft tissues of the mesocarp where the infection starts (Quillect et al. 1984). The embryo can facilitate the spread of the pathogen from the husk to the meat, through the germinative pore.

Other Hosts

Phytophthora palmivora is known to be the causal organism for many diseases of economically important tropical crops, such as black pod and stem canker of cocoa (*Theobroma cacao* L.), root rot and fruit rot of papaya (*Carica papaya* L.), and foot rot of black pepper (*Piper nigrum* L.). *Phytophthora palmivora* has also been isolated from orchids, durian (*Durio zibethinus*) and rubber. These crops are all grown in the Philippines and perform well in areas suitable for coconut growing. Durian and cocoa are economically important intercrops of coconut, with a coconut–durian mixed cropping system reported to be a profitable agricultural venture in Mindanao. However, it remains to be seen what influence intercropping of susceptible host plants will have on the severity of disease caused by *P. palmivora*. Attempts to establish an integrated disease-management system for phytophthora in a coconut-based farming system are the focus of our current research efforts.

Distribution of Bud Rot in the Philippines

Nationwide bud rot cases

To determine the extent and damage caused by the pathogen nationwide, disease surveys were

conducted in the main island groups of Luzon, Visayas and Mindanao in 1992. To obtain sufficient data, two methods were adopted: (i) disease incidence reports from PCA Regional offices were consolidated; and (ii) direct farm visits were undertaken. The highest disease incidence was observed in Mindanao and mostly in areas planted to MAWA (Table 6.2.1). Bud rot incidence in local populations of coconut were reported only in elevated areas such as Mt Banahaw in Luzon, and Camiguin Island in Misamis Oriental. In 1977–78, in an effort to minimise disease spread in infected areas, PCA launched a 'cut and burn' operation on the foot slopes of Mt Banahaw, covering Laguna and Quezon. An estimated 35,000 trees infected with bud rot were felled in 1977 (N. Bondad, Assistant Manager, PCA-Region IV-A, pers. comm.), with similar operations carried out in Camiguin Island in 1985 (J. Lopez, Agriculturist II, PCA-Camiguin, pers. comm.). The yearly data on bud rot cases in pilot PHFs was analysed, revealing a high incidence of the disease in areas of Mindanao where large MAWA plantings occurred (Figure 6.2.1).

Disease assessment

As a result of the data compiled in 1978–1985, disease mapping in the high-incidence Mindanao area was carried out. In the plantings of AADC and CII, bimonthly farm visits and disease monitoring found high levels of infection in the areas planted to MAWA. The highest disease incidence was found in AADC, where 3269 palms (12.6%) succumbed to the disease in a 600 ha MAWA plantation. In CII, the total bud rot cases recorded was 5559, an average disease incidence of 6.3%. Additional data on bud rot cases were collected in PCA research centres, where it was noted that disease incidence in mixed stands which included MAWA was as high as in areas planted to a single susceptible variety of coconut.

It has commonly been observed that bud rot infection of local cultivars is limited to the highlands due to the favourable climatic conditions for pathogen survival and disease development there. However, based on the high incidence of bud rot in MAWA PHFs nationwide, it was inferred that *Phytophthora* could infect the MAWA hybrid in all environmental conditions due to the hybrid's inherent susceptibility. In addition, it is noteworthy that the inherent susceptibility of West African Tall to *Phytophthora* was reported in Ivory Coast (Quillect et al. 1984).

The MAWA experience in the Philippines easily demonstrates the risk of large-scale plantings with a single or a few coconut hybrids where the plants may be inherently susceptible to a pathogen like *Phytophthora*. It is now appreciated in varietal

improvement programs that cultivars and hybrids recommended for replanting programs should be thoroughly and adequately evaluated in terms of their reaction to *Phytophthora* infection.

Disease symptoms

Visible symptoms

Bud rot of coconut is typically observed soon after a long dry season or after the occurrence of strong winds and typhoons. The first visible symptom of the disease is the drooping of the spear leaf, a symptom that can be easily recognised by a trained and experienced researcher or farmer. Infections initially causes the youngest or spear leaf of the coconut tree to wilt, while advanced stages result in the rotting or disintegration of bud or heart frond tissues, due to secondary infection by bacteria and other opportunistic fungi. As the disease progresses, the spear dries up completely with drooping of the young leaves becoming noticeable (Figure 6.2.1). At this stage, the bud or the coconut heart is already rotted with degenerated tissues and emanates a distinct foul odour. The spear leaf can easily be pulled out but the other leaves are still intact. Existing nuts can continue to develop and mature for 6 months to 1 year, even though the bud has already rotted.

Infection process

Infection by *Phytophthora* can be observed by felling and dissecting a newly infected tree. At this stage, the spear leaf is still green but already starting to wilt with evident loss of turgor. When leaves are sequentially removed, circular, water-soaked

lesions can be observed on the smooth portion of the unopened leaf near the base of the youngest leaf frond (Figure 6.2.2).



Figure 6.2.1 Drooping of the spear leaf due to bud rot in MAWA hybrid coconut.

Typical symptoms of fruit rot or immature nutfall are conspicuous irregular patches on the epidermal surface of tissues of immature nuts. These appear as water-soaked lesions, brownish in colour, of varied size and with yellowish margins. The infected nuts can be mistaken for aborted nuts due to premature browning and immature nut fall (Figure 6.2.3). Quillec et al. (1984) observed similar symptoms on MAWA hybrids in the Ivory Coast and Indonesia. When the affected nuts were split open, they exhibited brownish husks and, in severe cases, the meat failed to develop completely. This may be due

Table 6.2.1 Disease distribution of coconut bud rot in 1992 on three major islands of the Philippines.

Main island	Province	Location	Coconut variety	Age group	Total number of palms per farm	No. of cases	Disease incidence (%)	
Luzon	Laguna	Liliw	Local	50	650	35	5.4	
		San Pablo	Local	35	9,000	300	3.3	
		Nagcarlan	Local	35	3,500	500	14.3	
		Majayjay	Local	50	500	30	6.0	
	Quezon	Dolores	Local	30	24,000	500	3.1	
		Lucban	Local	45	2,192	72	3.3	
	Batangas	Lipa City	Local	25	300	14	4.7	
		Lemery	Local	20	218	38	17.4	
		Calaca	Local	20	197	17	8.6	
Visayas	Leyte	Baybay	MAWA	15	558	22	3.9	
Mindanao	Zamboanga	PCA-ZRC	Various collections	20	6,017	39	0.6	
	Bukidnon	Don Carlos	Local	20	193	13	6.7	
	Cotabato	Lake Sebu	Local	15	239	79	30.5	
	Mis. Oriental	Medina	MAWA			556	29	5.2
		Camiguin Is						

to colonisation by the pathogen, which is known to produce enzymes that macerate the infected tissues (Akinrefon 1982).



Figure 6.2.2 *Phytophthora palmivora* lesion on the inner leaf sheaths of the bud of a MAWA hybrid coconut



Figure 6.2.3 Nut rot in MAWA hybrid coconut, caused by *Phytophthora palmivora*.

The initial penetration of the nut by the pathogen may occur through the spikelets, since it was noticed that infection usually starts from the perianth portion and progresses towards the apex of the nut. It is evident that the pathogen can establish itself in the husk, shell, meat and water, since it can be isolated from all of these parts.

Pathogenicity

Isolation in pure culture

Several studies were conducted to establish the host–pathogen interaction. *Phytophthora palmivora* was isolated from infected areas using baiting techniques and selective synthetic media. Cultures grown in V-8 juice agar produced mycelia and sporangia. Tissue baiting using cocoa pods also favoured mycelial growth and production of sporangia. *Phytophthora* isolates from both bud rot and fruit rot disease displayed no variation in cultural characteristics.

Pathogenicity studies

Six-month-old coconut seedlings were mechanically inoculated with a pure culture of *P. palmivora* isolated from infected buds. Inoculation resulted in the production of brownish lesions and drooping of young leaves, with white mycelial growth observed on the area of inoculation. *P. palmivora* was re-isolated from the seedlings 20 days after inoculation. The production of symptoms on inoculated seedlings and the re-isolation of the pathogen indicated the pathogenicity of *P. palmivora* on susceptible coconut host tissues, and showed an infection cycle of 8–15 days on seedlings under favourable conditions.

In the case of fruit rot, the ‘single drop’ technique was employed. A portion of a 6-month old healthy nut was pricked by a sharp pin, a drop of the *P. palmivora* inoculum was placed on the pinpricks and covered with Scotch™ tape to provide a humid environment (Figure 6.2.4). Lesions were seen to develop at the site of inoculation, with an average daily increment of 0.85 cm.

Inoculation of coconut fruit through the spikelets produced symptoms after 2 days. Lesion



Figure 6.2.4 Lesions on coconut infected with *Phytophthora palmivora*.

development seemed to be faster after the third day. It was noted that 4-month-old nuts are more sensitive to infection than younger or almost mature nuts (Figure 6.2.5). In these studies, production of secondary sporangia resulting from the primary inoculum occurred within 72 hours of the time of inoculation.



Figure 6.2.5 Five-month old coconut artificially infected with *Phytophthora palmivora*.

Cross inoculation test

In order to determine the relationships of the *Phytophthora* isolated from buds and nuts, cross inoculations were performed. Isolates from the bud were used to inoculate the nut, and isolates from the nut used on the bud. Seedlings inoculated with the immature nutfall isolate displayed symptoms 30 days after mechanical inoculation, and nuts inoculated with the bud rot isolates showed symptom development after 5 days. Based on the size of the lesions that developed on the inoculated portion of the nut, the results suggest differences in the degree of specificity of different parts of the host plant are insignificant.

Bud rot observations in the germplasm collection

The Zamboanga Research Center (ZRC) of PCA maintains the largest collection of coconut germplasm in the world, with 83 cultivar collections and 42 hybrids for use in breeding programs and in genetic conservation. The average annual rainfall in this region is 1600 mm, falling predominantly between May and November, followed by a number of distinct dry months. The earliest incidence of bud rot and fruit rot disease in the germplasm plots were observed in 1986 and noted to be prevalent among the dwarf cultivars. The data collected indicate the greater susceptibility of the dwarf cultivars to nut fall and bud rot diseases, particularly the MRD and the MYD varieties, when compared to the tall and the local hybrids (Table 6.2.2). Interestingly, the

incidence of bud rot in MAWA plots was negligible during the observation period. This observation can be attributed to the heterogeneity of the populations planted in ZRC, which limits the continual spread of the disease, and to the environmental factors (warm temperature, high relative humidity and soil moisture, and the absence of typhoons and strong winds) that can trigger infection development and pathogen dissemination. Appropriate cultural management and immediate 'cut and burn' of infected trees in the collection plots was conjectured to prevent disease spread and minimise disease incidence on the MAWA plots.

The first cases of fruit rot were observed on the Red Cameron Dwarfs in 1986, while the MRD and MYD populations were found to be infected later. Fruit rot was observed to be severe, with about 5% of the total nuts succumbing to infection (Table 6.2.3). Emasculated palms showed especially high levels of rot, which could be attributed to contaminated cutting tools having been used. The emasculation activity was temporarily stopped and routine, 6-monthly treatments of Ridomil by root infusion (20 mL of 1.6 g a.i./tree) were undertaken. Monitoring has shown a reduction in disease incidence since that time.

Varietal Nut Reaction to Artificial Inoculation

Two varieties/cultivars, Malayan Red Dwarf (RMD) and Malayan Yellow Dwarf (MYD), and the locally developed hybrid PCA 15-1 (Catigan × Bago-Oshiro Tall), were tested for reaction to *P. palmivora* through mechanical inoculation using the 'single drop' technique. The results showed that MRD was subject to the most rapid increments in lesion size, while PCA 15-1 had the slowest. The reaction of MYD was not significantly different from MRD. Cross-variety inoculations were trialled, using detached infected nuts from one variety as a source of inoculum with which to inoculate healthy nuts from a second variety. This technique allowed us to identify a source of inoculum that produced the most consistent pathogenic results under field conditions, and can be used to test the susceptibility and resistance of potential parent material in breeding programs. The degree of resistance or susceptibility of the infected nuts was assessed as lesion expansion over time. The increase in size and depth of the lesion were measured daily using calipers. Infected MAWA nuts used as source of inoculum to inoculate healthy MRD nuts produced symptoms similar to those observed in the field.

The initial results of the varietal nut reaction could be used in evaluation studies to determine the

performance of promising hybrids in terms of disease reaction. This study itself has already provided information on promising parental materials for hybridisation programs and in determining sources of resistance. The significant resistance to infection displayed by the local hybrids, which were produced from local dwarf and local tall cultivars, indicates that the local tall parent cultivars could be sources of parental genes with possible inherent resistance to *Phytophthora* infection.

As reflected in Table 6.2.3, significant differences in reaction to the disease were found among dwarf and tall cultivars. When artificially inoculated, the red and yellow-pigmented cultivars were found to be highly susceptible when compared to the green-pigmented cultivars, and in particular when compared to the local populations, thus confirming field observations. The results of the inoculation tests show that sources of resistance to *Phytophthora*

infection can be determined, which is vital in the process of selecting promising cultivars for replanting programs, and in the formulation of control strategies to contain the disease.

Recommendations

- Collaborative efforts among breeders and pathologists are needed in breeding programs to look beyond improving agronomic characters of the hybrids to be developed while at the same time also including resistance to major diseases.
- Comprehensive assessment of recommended cultivars and hybrids for distribution and replanting is imperative to assure disease-free or disease-resistant planting materials.
- To minimise losses from the disease, areas identified as having high inoculum levels of *Phytophthora* should be avoided in planting

Table 6.2.2 Bud rot cases at the germplasm collection at the Zamboanga Research Center of the Philippine Coconut Authority.

Population	Number of palms	Bud rot incidence 1991		Bud rot incidence 1992	
		No.	%	No.	%
CAT × LAG hybrid	190	1	0.6	0	0.0
CRD × WAT hybrid	130	1	0.8	0	0.0
CAT × BAY hybrid	168	0	0.0	1	0.6
MYD × WAT hybrid	401	3	0.7	1	0.2
MRD × TAG hybrid	22	0	0.0	3	13.6
MAT × MYD hybrid	150	0	0.0	1	0.7
BAO × CRD hybrid	90	1	1.1	0	0.0
BAY × CRD hybrid	90	1	1.1	0	0.0
RNL × GDS hybrid	120	1	0.8	0	0.0
TAG × WAT hybrid	120	0	0.0	1	0.8
TAG × RCD hybrid	60	0	0.0	1	1.7
Aromatic dwarf	137	0	0.0	1	0.7
Catigan dwarf	1115	0	0.0	3	0.3
Banigan	96	0	0.0	1	1.0
Galas	110	0	0.0	1	0.9
RNL-A tall	565	1	0.2	0	0.0
Magtuod dwarf	134	1	0.7	2	1.5
MRD dwarf	488	0	0.0	4	0.8
MYD dwarf	1557	0	0.0	3	0.2
Macapuno	96	3	3.1	0	0.0
Agta tall	84	2	2.4	0	0.0
SNR tall	134	0	0.0	1	0.7

Table 6.2.3 Fruit rot incidences at the germplasm collection in PCA-ZRC (1991–1992).

Population	Number of palms	Number of palms infected	Number of bunches infected	Number of nuts infected
MRD	488	26 (5.3%)	42	301
MYD	1557	7 (0.5%)	15	88
Buswang	90	1 (1.1%)	4	34

susceptible coconut cultivars and intercrops known to be infected by the pathogen.

- Planting of homogeneous varieties/populations in environments that may favour disease development should be discouraged to avoid disease epidemics.
- Adoption of proper cultural management and proper disposal of infected palms and plant parts is essential to eliminate possible sources of pathogen and control the spread of disease.

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6.3 Distribution and Progression of *Phytophthora* Bud Rot Disease of Coconut in Selected Areas in the Philippines

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Abstract

Geographical distribution of *Phytophthora* bud rot on coconut in the Philippines was determined from 1990 to 1999 through a survey in areas with reported incidence of the disease. Records of the disease reached to 4.1%. Over 11,000 palms were killed by *P. palmivora*, with the three provinces of Davao (Davao del Norte, Davao del Sur and Davao City) having the highest incidence. It was found that the disease infected several coconut cultivars all over the country. The Malayan Yellow Dwarf × West African Tall (MYD × WAT) hybrid (known as MAWA) introduced into the country was the most susceptible, with an incidence rate of 2.7%. Most of the affected palms were 3–15 years old with MYD or Malaysian Red Dwarf (MYD) parentage. Studies on the spatial and temporal distribution of the disease showed that it occurred in multiple foci that were distributed throughout the experimental area. It had both the regular and contagious or cluster-distribution pattern. The disease progress curves suggest that bud rot follows a continuous ‘compound interest’ model. It follows that the progress of the disease at any given time is a function of the initial inoculum and the number of effective contact points between a susceptible host and inoculum per unit time. Analysis of the infection rates using logistic growth model in three observation sites gave rates of 0.065, 0.074 and 0.157 per unit per year in MYD × WAT, Laguna Tall and MYD × Hijo Green Tall (HGT), respectively. Regardless of genotype, infection rate was established at 0.228 per unit per year.

Introduction

The occurrence of bud rot disease of coconut in the Philippine provinces of Laguna and Quezon was first reported by Copeland (1908). A decade later, Reinking (1919) identified *Phytophthora faveri* Maubl. (also *P. palmivora* Butler) as the causal organism of coconut bud rot, following an extensive study of its morphology, including growth in various media, mycelium, conidiophores, conidia, chlamydospores and absence of sexual bodies. Bud rot is characterised by the wilting of the spear leaf due to the rotting of the bud (Figure 6.3.1). The fungus has infected thousands of coconut palms since it was first identified in the Philippines.

However, a thorough investigation of its the mode of spread, rate of infection and geographical distribution was made only in 1989 to 1998, led by the Crop Protection Division, Davao Research Center, Philippine Coconut Authority (PCA). The information generated about behaviour of the disease relative to cultivar, age of the palm, location and climatic conditions prevailing in the growing area is vital in the management of the disease.

Methodology

Disease distribution and assessment of bud rot incidence

Disease surveys and mapping were conducted on coconut farms in the Philippines, in the provinces of Laguna, Quezon, Batangas (Luzon), Leyte and Samar (Visayas), Zamboanga, Bukidnon, Misamis Oriental,

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South Cotabato, Camiguin Island, Davao del Sur, Davao City, and Davao del Norte (Mindanao). This survey was conducted in collaboration with extension staff of the Field Operation Branch of PCA. Before the survey, a training course on disease identification and basic control methods was given to the Coconut Development Officers and several farm leaders nationwide. Data on the incidence of bud rot cases were reviewed and consolidated annually from 1990 to 1999.



Figure 6.3.1 (Upper left) The initial symptoms of bud rot: early wilting of the spear leaf. (Upper right) Abnormal hanging and desiccation of the spear leaf, another bud rot symptom. (Lower left) Rotted tissue shows as purple to pale pink, and has the consistency of soft cheese. (Lower right) A dissected bud showing internal rotting of the tissue. The rotten tissue emits the odour of putrefaction.

Data on bud rot incidence were collected from different experimental plots planted with various coconut hybrids/cultivars at the PCA's Davao Research Center at Bago-Oshiro, Davao City and the Zamboanga Research Center, as well as at different multi-location sites of the Breeding and Genetic Division for at least 5 years.

The following data were gathered in each area surveyed:

- cultivar/hybrid
- palm age
- number of palms
- number of infected palms

- percent of disease incidence computed as the number of infected palms
- disease incidence per cultivar.

Disease mapping of bud rot

Actual mapping of disease spread was done in at least 10 ha per planting area, with approximately 1000 coconut palms and at least 10% disease incidence. These were established in Payahan, Camiguin Province, Ayala Agricultural Development Corporation, Darong, Davao del Sur and Conception Farm in La Filipina, Davao del Norte. With the use of farm maps indicating the distribution of coconut palms, the exact location and number of diseased palms were recorded.

Data on disease incidence were collected every 3 months. In addition, rainfall data within the period of observation were gathered. The increase in disease incidence was expressed as the number of infected palms divided by the total number of palms, calculated annually in each experimental area.

Disease plant density distribution analyses

To determine the spatial pattern of bud rot distribution in the three experimental sites, a local density distributions diseased plants were compared with expected random distribution. The mean (\bar{x}) and variance (s^2) of the diseased palms to the total local population at each site were taken and the goodness of fit was tested using the chi square (χ^2) distribution parameter. In events where variance is equal to the mean, the population is said to be randomly distributed. If the variance is less than the mean, then the distribution is regular. Computations were made following the formula of Gomez and Gomez (1984).

Disease progress curve

Disease progress curves for each experimental area or cultivar were determined by plotting the disease proportion against time, using the data gathered from 1990 to 1999.

Infection rate

Infection rate per site per cultivar was calculated using the same data as for the disease progress curve. Infection rates were estimated from the logistic growth model described by Van Der Plank (1963), using the differential equation:

$$dY/dt = rYt(1 - Yt)$$

where the change in proportion of disease Y , with time t , is equal to the rate of infection r , multiplied by the proportion of the disease at any given time and then multiplied by a correction fact or $(1 - y)$. Disease

proportion was transformed according to the disease growth model and regressed with time. The infection rate, which was the slope of the line, was determined.

Results and Discussion

Geographical distribution and assessment of bud rot incidence

Geographical distribution

Figure 6.3.2 shows that bud rot disease is widely distributed throughout the country where coconut is grown. In Luzon, Laguna, Quezon and Batangas, the number of bud rot infected palms was 893, 654 and 69, respectively, during the 10 years of observation. Areas surveyed in these provinces are situated at high elevations where low temperature and high moisture favour disease development. In Visayas, where only the province of Leyte was visited, 22 infected palms were found in a 5 ha coconut farm. Disease severity was highest in Mindanao. Several provinces were affected but the incidence was highest in the three provinces of Davao, with Davao del Sur having 5224 bud rot infected coconut palms, Davao del Norte 1749, and Davao City 1163.



Figure 6.3.2 Locations in the Philippines where bud rot disease of coconuts was found.

Considering the country as a whole, Mindanao had by far the largest proportion of total disease incidence, 85.2%. Areas in Luzon areas had 14.6% of the total percentage disease incidence, while Visayas had only 0.2%. Average disease incidence across the country reached 4.1%, which translates to 11,130 palms killed in our experimental survey plots over the 10 years of observations.

Assessment of bud rot incidence by cultivar/hybrid

Among the dwarf cultivars, the highest disease incidence was observed in Malaysian Red Dwarf (MRD) (13.7%). Among the tall cultivars, Laguna Tall (LAGT) had the highest incidence (5.6%) followed by Hijo Green Tall (HGT) (5.2%).

Among the hybrids planted in different places in the country, disease incidence was relatively higher in Malayan Yellow Dwarf × West African Tall (MAWA) hybrid (4.4%) plantings than in the local cultivars (Table 6.3.1). It should be noted that almost all areas surveyed with LAGT plantings were located in the highlands, where relative humidity is high, a critical factor that predisposes coconut palms to pathogen infection. The MAWA hybrids, on the other hand, have been used for massive planting both in high and low-lying areas of the country. It was also observed that hybrids with MRD or Malayan Yellow Dwarf (MYD) as one of the parent materials had a higher disease incidence than the other hybrids. This observation is supported by the data gathered in the multi-location trial sites of PCA. Catigan, a local dwarf cultivar, was observed to be quite tolerant to the disease.

Assessment of bud rot disease incidence by age group

Table 6.3.2 shows the effect of coconut age on the incidence of bud rot. Generally, the incidence of the disease falls in mid-aged palms, but then rises again in older trees. Coconut palms ranging in age from 3–10 years were more susceptible to bud rot with disease incidence of 4.3% or total disease occurrence of 4982 bud rot cases. This was followed with palms ranging in age from 11–15 years (2569) or 3.9% disease incidence. Coconut palms ranging in age from 41–50 years had the highest incidence (657) of bud rot infection. This trend might have something to do with the physiology of the coconut bud as it matures. As Mackenzie et al. (1983) indicated, the phenomenon of adult plant resistance may in some cases be explained by age-specific changes of the plant.

Mapping of bud rot incidence

Among the three areas, the AADC coconut plantation at Darong Davao del Sur, where MAWA was planted, had the highest bud rot occurrence with 0.37, followed by La Filipina, planted with MYD × HGT with 0.24. LAGT planted in Camiguin province had the least incidence at 0.13 (Table 6.3.3). Once again, MAWA and hybrids with MYD parentage show a significant degree of susceptibility to *Phytophthora* infection.

Table 6.3.1 Disease distribution of bud rot by coconut cultivar/hybrid.

Genotype	Age group (year)	Total number of palms	Disease incidence	
			Number	%
Dwarf				
Catigan	20	971	1	0.1
Malaysian Red Dwarf (MRD)	14	110	15	13.7
	Total	1,081	16	1.5
Tall				
Baybay	12	288	7	2.4
Hijo Green Tall	12	96	5	5.2
Laguna Tall	20- 30	57,623	3,227	5.6
Tagnanan Tall	12- 18	33	271	0.8
	Total	91,703	3,510	3.8
Hybrid				
	12	96	2	2.1
CAM × BAY	20	53	3	5.7
CAT × BAO	20	70	2	2.9
GDH × WAT	12	384	8	2.1
MRD × BAY	12	384	16	4.2
MRD × HGT	12	576	16	2.8
MRD × RIT	12	480	12	2.5
MRD × TAG	12	4,500	60	1.3
MYD × HGT	12	192	5	2.6
MYD × RIT	10-20	168,429	7,357	4.4
MYD × WAT	12	73	1	1.4
NRC × WAT	12	96	7	7.3
PGD × LUP	12	96	7	7.3
TAC × BAO	12	96	2	2.1
WAT × RIT	Total	175,525	7,498	4.3

Table 6.3.2 Disease distribution of bud rot by age group.

Age group	Number of palms	Disease incidence	
		Number	%
03 - 10	115,757	4,982	4.3
11 - 15	65,920	2,569	3.9
16 - 20	39,354	673	1.7
21 - 30	30,929	1,253	4.1
31 - 35	13,924	996	7.2
41 - 50	8,342	657	7.9
Total	274,326	11,130	4.1

Table 6.3.3 Cumulative disease proportion of bud rot disease.

Location	Genotype	Number of palms	Disease incidence							
			1990	1991	1992	1993	1994	1995	1996	1999
AADC, Darong, Davao del Sur	MYD × WAT	1144	0.13	0.14	0.17	0.17	0.20	0.23	0.24	0.37
La Filipina, Tagum, Davao del Norte	MYD × Hijo Tall	911	0.05	0.08	0.08	0.08	0.10	0.10	0.11	0.24
Payahan, Camiguin Island	Laguna Tall	1025	0.07	0.08	0.09	0.09	0.10	0.12	0.13	0.13

Diseased plant density distribution analysis

The spatial distribution of bud rot in the different experimental areas over a 9-year observation period is presented in Figures 6.3.3–6.3.5. Initial descriptive patterns of the disease indicate that it is distributed throughout the entire plots and has multiple foci. The randomness of the disease distribution was analysed using the theoretical binomial distribution under the random distribution analysis. Table 6.3.4

shows that variances of the majority of the areas throughout the observation period are greater than the means, an indication that the disease distribution is continuous or clustered.

Multiple foci were observed and the disease progressed from one infected palm to the next. This observation follows that of Steer and Coastes-Beckford (1990). Mackenzie et al. (1983) also reported that dispersal mechanisms of the

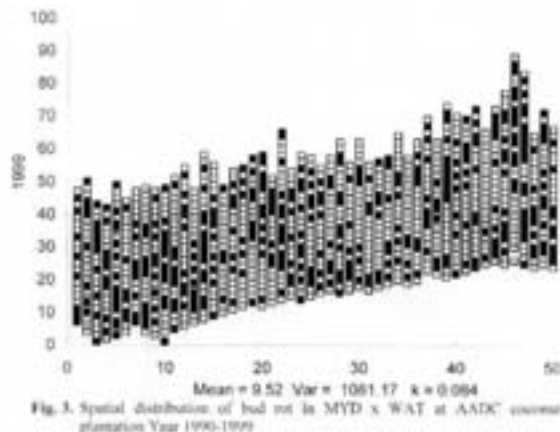
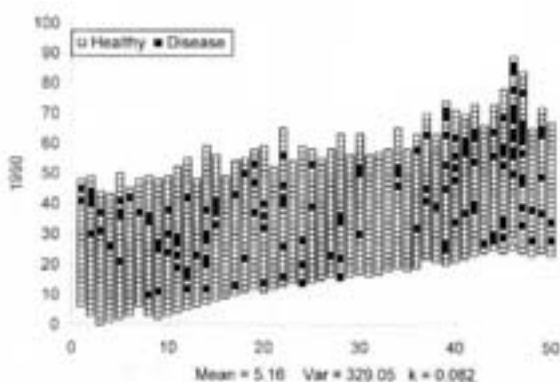


Figure 6.3.3 Spatial distribution of bud rot in MAWA hybrid at AADC coconut plantation year 1990–1999.

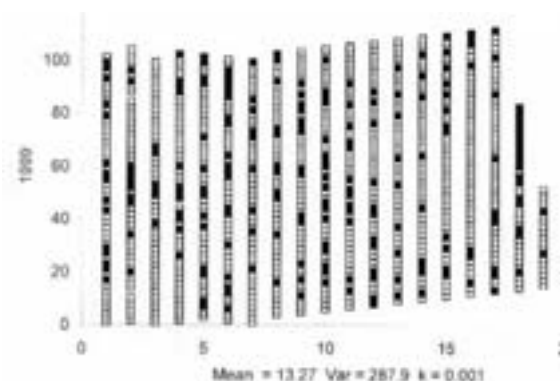
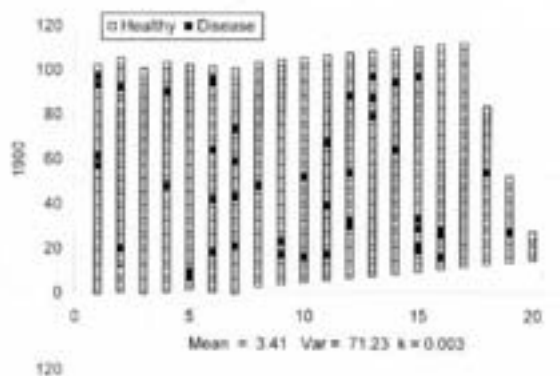


Figure 6.3.4 Spatial distribution of bud rot in MYD × Hijo Green Tall at La Filipina coconut plantation year 1990–1999.

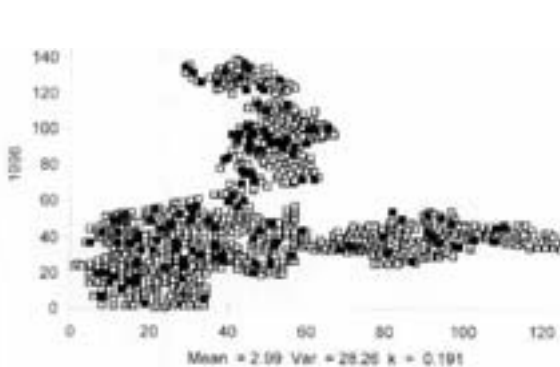
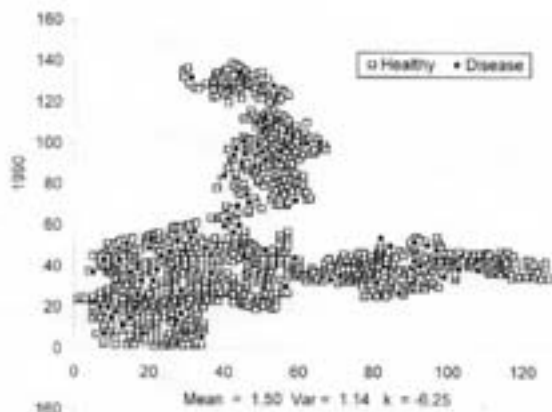


Figure 6.3.5 Spatial distribution of bud rot in Laguna Tall coconut palms at Camiguin Island. 1990-1999.

inoculum of *Phytophthora* spp. are closely or directly related to water. Rain splash, among other water movements, may account for local dispersal within canopies of the palm, moving the infectious spores between different palm trees and different fields and areas.

Disease progress curve

Progress of bud rot disease in the three sites during the 9-year observation period was determined by plotting the disease incidence over time. Figure 6.3.6 shows a disease progress curve that appears to follow the compounded continuous interest (CCI) type described by Van Der Plank (1963). CCI curves or epidemics, according to Van der Plank, have the potential for exponential explosion, sometimes resulting in catastrophic disease.

Infection rates

Apparent infection rates (represented by r values) are estimates of how fast an epidemic progresses over time when adjusted for multiple infections. They are calculated as linear regression coefficients of the logit-transformed disease proportions (Mackenzie et al. 1983). Table 6.3.5 and Figure 6.3.7 show the apparent infection rates of bud rot in the three experimental areas planted with different cultivars ranged from 0.157 to 0.065 per unit per year. The area with the highest apparent infection rate of 0.157 per unit per year is the MYD × HGT plantations at La Filipina. According to Mackenzie et al. (1983), cultivars differ in their apparent infection rates, which may be due to different levels of horizontal resistance. Regardless of genotype and area, infection rate is 0.228 per unit per year.

Table 6.3.4 Analysis of bud rot disease distribution in coconut palms at three experimental sites in the Philippines.

Area	Total population	Year	Disease incidence (no. of cases)	Mean (X)	Variance (S ²)	Aggregation index (K)
AADC	1144	1990	144	5.17	329.05	0.0824
		1991	164	5.50	438.80	0.0698
		1992	192	5.65	413.38	0.0770
		1993	197	5.87	442.96	0.0789
		1994	230	6.75	711.88	0.0647
		1995	266	7.06	706.71	0.0713
		1996	274	7.14	708.65	0.0260
		1997-99	426	9.82	1081.17	0.0845
La Filipina	911	1990	46	3.41	71.23	0.0031
		1991	69	5.09	195.59	0.0017
		1992	72	5.22	196.51	0.0017
		1993	76	5.29	194.91	0.0017
		1994	87	5.73	212.11	0.0016
		1995	95	5.92	256.78	0.0013
		1996	98	5.95	259.69	0.0012
		1997-99	222	13.27	287.95	0.0010
Camiguin	1025	1990	75	1.50	1.14	-6.2500
		1991	86	1.69	8.30	0.4358
		1992	88	1.68	8.05	0.4437
		1993-94	97	1.87	15.15	0.2636
		1995	122	1.99	19.61	0.2252
		1996	123	2.23	28.26	0.1905

Table 6.3.5 Simple linear regression analysis, using a logistic model, of progress of bud rot disease in three different locations in the Philippines.

Location	Genotype	Intercept	Apparent infection rate (r)	R-squared
AADC, Darong, Davao Del Sur	MYD × WAT	-301.900	0.065	0.982
La Filipina, Davao Norte	MYD × Hijo Tall	-377.900	0.157	0.925
Camiguin Island	Laguna Tall	-220.300	0.074	0.925
	Average	-420.600	0.228	0.894

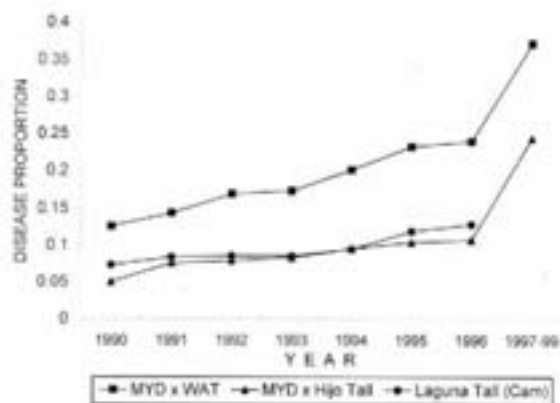


Figure 6.3.6 Disease progress curve of bud rot of coconut in AADC, La Filipina Plantation in Davao Sur and Norte and Camiguin Island 1990–1999.

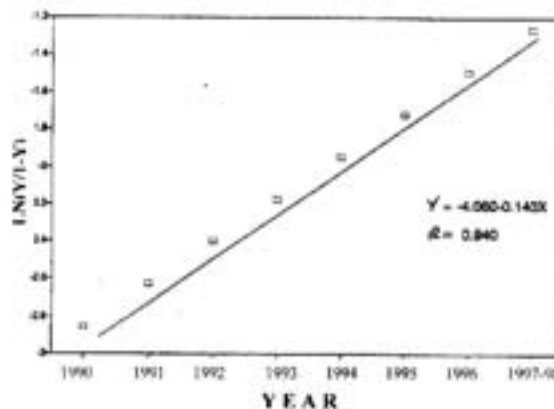


Figure 6.3.7 Regression line showing the relationship of transformed disease progress to time.

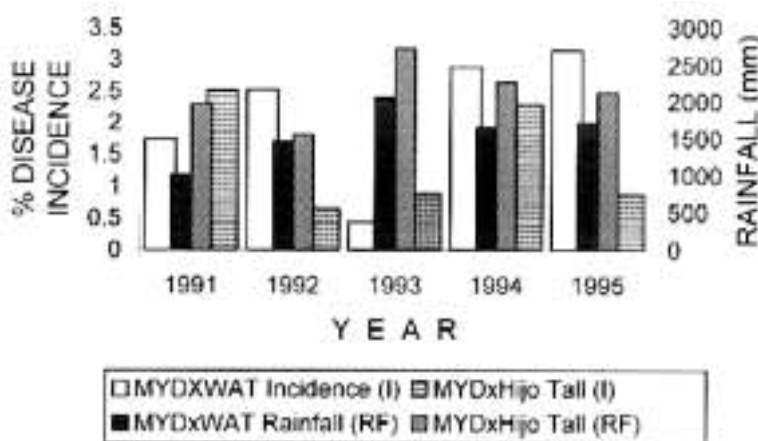


Figure 6.3.8 Incidence of bud rot in MYD × WAT and MYD × Hijo Tall coconut palm hybrids.

Effect of rainfall on bud rot incidence

Rainfall increased the incidence of disease in the MYD × HJT hybrid in La Filipina but not with the MAWA hybrid in AADC. This might be related to high relative humidity in the area.

Infection by *P. palmivora* on coconut occurs when relative humidity is higher than 94% and the temperature is below 24°C. It might be inferred from the inverse relationship of bud rot incidence to rainfall in MAWA plantation at AADC (Figure 6.3.8) that disease development in this area is not largely dependent on climatic conditions, particularly rainfall, but rather on the susceptibility of the MAWA hybrid.

Conclusions and Recommendations

Bud rot is indeed a major fatal disease of coconut palms in the Philippines. It is widely distributed, has the ability to infect several, if not all coconut genotypes, and most important of all, it has the potential for exponential growth, an occurrence that would will be catastrophic to the coconut industry.

The establishment of the apparent susceptibility of hybrids with MYD and MRD as parent materials is important information and such materials are to be avoided by breeders in breeding *Phytophthora*-resistant cultivars/hybrids.

Establishing the pattern of disease spread is vital in framing recommendations for preventive control measures. Based on the results of this study, it is recommended that preventive measures such as sanitation (cutting and burning of affected palms) and fungicide application be applied to neighbouring palms in infected areas to prevent further spread. Monocropping with highly susceptible cultures such as MAWA is to be discouraged. Also, genetically uniform planting leads to continuity of spread of the disease, leading to outbreaks.

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6.4 *Phytophthora capsici* on Black Pepper in Indonesia

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Abstract

Foot rot of black pepper (*Piper nigrum* L.) is an important constraint to production of pepper in Indonesia and many other parts of Southeast Asia where pepper is grown. Cultivation practices and the intensity of management is dependent on the highly variable price of pepper. This chapter summarises the symptoms of the disease and describes its epidemiology, and provides an outline of the options for disease control.

Introduction

Phytophthora capsici Leonian causes the most destructive and economically significant disease of black pepper (*Piper nigrum* L.). *P. capsici* attacks all parts and growth stages of the black pepper plant. The disease, which was first reported in Lampung in 1885, has been called foot rot disease since 1928 (Muller 1936). The causal agent was first identified as *P. palmivora* var. *piperis* (Muller 1936), and later determined as *P. palmivora* MF4 (Tsao et al. 1985). Later still, it was renamed *P. capsici sensu lato* (Tsao and Alizadeh 1988). The disease is now found in almost all pepper grown in Indonesia.

Pepper (black and white) is the seventh largest export income earner for Indonesia. The total area under pepper cultivation is about 136,450 ha, and the activity involves over 130,000 farmers. Smallholders conduct almost all pepper cultivation in Indonesia. They have limited access to capital, and fully manage their cultivations only whenever the pepper price is high, abandoning them if the price falls. They usually use systemic fungicides to control foot rot disease, to which all cultivated pepper varieties grown in Indonesia are susceptible.

Lampung and Bangka are the main black pepper producing areas. Foot rot disease destroyed the pepper area in Lampung before the second world war, while in Bangka, the disease damaged about 32% of pepper plants in 1965. The other pepper areas are in West, Central and East Kalimantan.

We collected 168 *Phytophthora* isolates causing foot rot. The resulting population of *P. capsici* consisted of 148 A1 mating type isolates and 20 A2 mating type isolates. Both mating types were found in Lampung and Kalimantan, while in Bangka only the A1 mating type was found. Among those isolates, 43 were morphologically and physiologically characterised. The results showed that all isolates were *P. capsici* except one, which was identified as *P. nicotianae* (Manohara and Sato 1992).

Disease Symptoms

The first symptom of foot rot is a slight wilt of the vine. The leaves become pale and the vines droop (Figure 6.4.1). At this point, the leaves may fall prematurely, puckering along the edges and becoming yellow before they fall. Occasionally, necrosis is observed at either end of the leaf. After defoliation, the fruit begins to wrinkle and dry out. The flower spikes and lateral stems become necrotic and break off at the nodes. The post holding the vine is left bare of all but the three climbing stems. The decline of the vine is rapid, 75% of the leaves may fall within 7–14 days of the first signs of wilt. The wilting is caused by the destruction of the underground parts of the main stem, although the

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root, collar, leaves, flower spikes and fruits are also susceptible to attack. Complete destruction of the main lateral roots and girdling of the stem at the crown cause the wilt. In some cases, collar rot may occur rapidly at the base of the plant, so there is no time for the leaves to abscise and drop. This, so called sudden-death, leads to dead plants with all the leaves still attached. Infected leaves are found on the lower foliage close to the mound below the vine. Necrotic lesions are observed on the leaves. These may be circular and deep brown in colour, with a distinct fimbriate edge. Fimbriate lesions are diagnostic of foot rot. They tend to occur on younger leaves; the fimbriate edge becomes less distinct when the infection becomes less active in drier weather. Concentric rings may appear around the lesions after continued wet weather. Stems can also become infected, showing water-soaked patches. The vine may become locally defoliated near the site of stem infection. Dieback of the stem can occur as the infection progresses along the vine. It is more difficult to isolate *P. capsici* from infected roots and stems than from leaf lesions. Below-ground symptoms are sometimes detectable at the first sign of wilt. Vines older than 3 years seem to be the most susceptible to foot rot (Holliday and Mowat 1963; Erwin and Ribeiro 1996). The A1 types isolated from Lampung and East Kalimantan are more pathogenic than A2 type. Conversely, the A1 type isolated from West Kalimantan is less pathogenic than A2 type.



Figure 6.4.1 Foot rot in pepper, caused by *Phytophthora capsici* and giving rise to pale leaves and drooping of the vine (plant on the right).

Disease Epidemiology

The principal source of inoculum of *P. capsici* is infected plant debris. Leaves are infected by

inoculum splashed up from the soil. The severity of foot rot increases during periods of rainfall in the monsoon season, and when day and night temperatures vary between 19 and 23°C (Erwin and Ribeiro 1996). Other predisposing factors include planting pepper in soils that are low in organic matter and nutrients such as calcium, magnesium, and potassium, but high in nitrogen (Nambiar et al. 1965). Vectors such as termites and slugs can transport inoculum within and between vines (Erwin and Ribeiro 1996).

Soil moisture is one of the most important environmental factors for the survival of *Phytophthora*. Propagules of *P. capsici* (isolated from Lampung) survived for more than 20 weeks in Latosol soil at 100% field capacity. The fungus survived as a saprophytic stage on pepper leaves for 11 weeks in soil at 60–100% field capacity, while on the stem survival time fell to 8 weeks (Manohara 1988).

P. capsici infects leaves close to the soil surface, usually after heavy rain at the start of the wet season. Penetration by zoospores occurs 4–6 hours after interaction. There are two methods of infection: direct penetration through epidermis, and indirect penetration through stomata. Brown–black minute spots appear 18 hours after infection (Manohara and Machmud 1986). Collar infection causes sudden wilting, the leaves turn brown–black and dry while they are still attached to the plant.

Disease Control

The first step in preventing the disease is to plant on well-drained sites not planted to black pepper for at least a year beforehand. Removal of diseased vines, followed by application of a copper-based fungicide around the diseased roots to prevent spread to other vines is highly recommended. Bordeaux mixture has been reported to be effective, as have metalaxyl and fosetyl-A1 when applied to the foliage (Erwin and Ribeiro 1996). There is limited resistance to foot rot in *P. nigrum* and other species of *Piper* (Sitepu 1993), but some success has been achieved in using disease-tolerant species as rootstocks for current cultivars (Manohara et al. 1991). Application of metalaxyl as a root soil drench has been used to control root and stem rot in black pepper (Erwin and Ribeiro 1996). Application of fungicides is recommended at the beginning of the wet season, with follow-up sprays at 7–10-day intervals (Sitepu 1993). Another successful disease-control method developed in Sarawak uses root infusion of phosphorous acid, as described in chapter 7.4. There are some cultural practices that can minimise the impact of foot rot

disease. These include weeding around the bases of the vines to discourage the build-up of moisture that can encourage the proliferation of inoculum, and pruning the lower canopy to prevent it from coming into contact with soil-borne inoculum. However, clean weeding usually done by farmers may in many cases cause faster disease spread than limited weeding. Improving soil drainage also discourages disease development. In areas where *P. capsici* is endemic, rows of black pepper should be alternately planted with a perennial crop that is resistant to foot rot. The application of organic waste matter (such as trash from maize, rice, mungbean, peanut or soybean crops) to the soil can encourage the development of microorganisms that are antagonistic to *P. capsici*. The eradication and burning of infected vines is also highly recommended (Sitepu 1993).

An integrated approach is needed to control foot rot in pepper. This will include introduction of adequate drainage systems, limited weeding, fertilising of the pepper plants at recommended dosages and times, pruning the lower branches of pepper plant, especially during rainy season to reduce humidity at the collar and prevent the lower leaves coming into contact with soils that might be infected by *P. capsici*, and the use of phytophthora-tolerant varieties. The use of tolerant varieties such as Natar 1 is recommended when farmers want to expand their plantings. Planting cover crops such as *Arachis pintoii* among pepper plants is believed to be better than clean weeding, as *A. pintoii* inhibits the dissemination of *P. capsici*. During the rainy season, it prevents splashing, onto the lower leaves, of soil particles that may be contaminated with *P. capsici*. Inorganic fertiliser (NPK) that contains more potash than nitrogen has also been reported as reducing *P. capsici* infection (Zaubin et al. 1995).

The amendment of organic matter such as rice straw, and maize, soybean, peanut and mungbean waste, reduced the disease intensity by about 20–50% (Kasim 1985). Root exudates of *Allium fistulosum*, *A. ascalonicum*, *A. schoenorapsum* and *A. sativum* have also been reported to inhibit zoospore germination. The rhizospheres of *Allium* spp. are suitable for the growth of some microbial antagonists such as *Trichoderma* spp. and fluorescent bacteria, and the planting of these species around pepper plants is therefore recommended (Manohara et al. 1994).

Trichoderma harzianum Rifai (BLT 1), in the form of substrate or a pelletised formulation, has shown good potential for control foot rot disease. Incorporating it with some organic materials has been shown to reduce the severity of foot rot disease

by up to 50% in greenhouse tests (Manohara and dan Wahyuno 1995).

Future Research

Introducing resistant varieties is an effective and economic way to control foot rot disease on black pepper. Even though black pepper is a perennial crop, it is commonly propagated vegetatively. Therefore, breeding programs for resistance can be accelerated through the rapid multiplication of resistant hybrid clones. Conventional and somatic hybridisation could be adopted in the production of such hybrids.

Currently, the management of foot rot in pepper is conducted without much knowledge of the population biology of the causal organism. Different mating types occur in the pathogen populations, and differences in the pathogenicity between isolates within and between may exist. Therefore, analysis of the structure of pathogen population should be initiated in parallel with screening for sources of resistance. Sources of genetic resistance have been identified in wild black pepper species such as *Piper hirsutum*, *P. aurifolium* and *P. cubeba* (Kasim 1981). Some varieties of black pepper showed tolerance to *P. capsici* infection. These included Natar I, Bangka, Pulau Laut, Merapin and Banjarmasin Daun Lebar. In order to select a number of competent strains, representing the diversity of pathogen populations in the field for use in selection for disease resistance, more research is needed to characterise the pathogen populations and gain further insight into the nature of the host–pathogen interaction.

Disease resistance alone has as yet not been able to halt the serious economic impact of foot rot disease in pepper. To control the disease, resistance therefore needs to be combined with other management practices in an integrated approach.

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6.5 Phytophthora Diseases of Rubber

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Abstract

Rubber is affected by a group of phytophthora diseases including pod rot, leaf fall, black stripe of the tapping panel, and stem or patch canker. Black stripe disease was the first noted in Sri Lanka and is widespread in Southeast Asia as well as Africa and America. Other phytophthora diseases are also common throughout most rubber-growing areas. Black stripe and leaf fall cause serious damage but economically important outbreaks are confined to areas with long periods of high rainfall. Although patch or stem canker is widespread, recent records of high economic impact are few. At least six species of *Phytophthora* have been reported to be associated with diseases of rubber. The most common species are *Phytophthora palmivora* (Butl.) Butl., *P. meadii* McRae and *P. botryosa* Chee.

Introduction

In the late nineteenth century, rubber was introduced from South America to Sri Lanka and later to Malaysia and other countries in Southeast Asia. By 1910 Asia had become the main supplier of natural rubber. FAO statistical records from 1990–1998 indicate that 6.9 million ha of rubber were planted in India and Southeast Asia including Indonesia, Malaysia, Myanmar, Sri Lanka, Thailand and Vietnam. The major rubber-grower countries are Indonesia, Malaysia and Thailand, each with more than a million hectares.

Like most other cultivated crops, rubber is facing serious problems from several diseases, of which at least 40 have been reported. Among these, phytophthora diseases are affecting rubber in most growing areas. Infection occurs in most parts of the rubber tree including seedpod, leaf, leaf petiole, tapping panel, stem and trunk. However, there is no record of root disease caused by phytophthora in rubber. Black stripe, a disease of the tapping panel, was the first phytophthora disease to be recognised in Sri Lanka in the early 1900s. Later, pod rot, leaf fall, stem or patch canker were reported. The impacts of phytophthora diseases on rubber production are a reduction in latex yield, caused by

the panel and stem diseases, and a reduction in growth due to leaf fall. In addition, pod rot affects seed production for root stock propagation.

Prophylactic fungicidal spraying is extensively used to control phytophthora leaf fall in various parts of the world, including India, Malaysia and Sri Lanka. However, application of chemicals to control leaf fall from mature rubber trees is impractical and costly, due to the height of the trees and the large plot sizes. In contrast, disease control using fungicide is more effective and economically attractive to control black stripe and stem canker than leaf fall. In addition, clones that are tolerant to leaf fall – RRIM712, PR255, PR261 and GT1 – have been recommended and are replacing the highly susceptible rubber clones RRIM600 and PR107 in the areas conducive to disease development. Agronomic practices such as reduction of plant density and avoidance of excessively moist conditions by removal of vegetation are also recommended.

In this paper an attempt is made to summarise information regarding phytophthora diseases in rubber, with emphasis on disease incidences in the main rubber-producing countries in Southeast Asia.

Epidemiology

Annual occurrences of phytophthora leaf fall are common in India (Pillai et al. 1989), the southwest coast of Thailand (Kajornchaiyakol 1977, 1980), the

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northern and western states of Malaysia (Johnston 1989), and in Myanmar (Turner and Myint 1980) and Sri Lanka (Jayasinghe and Jayaratne 1996). In these regions the disease is most prevalent during the monsoon with long periods of high rainfall and constant high relative humidity (Wastie 1973). In Thailand, leaf fall epidemics occur during June–December (Pattanakul et al. 2001), and in Sri Lanka during May–September (Jayasinghe and Jayaratne 1996). In most cases, infection first takes place on immature pods, giving rise to pod rot, which then acts as a source of inoculum to fuel the leaf fall epidemic (Pattanakul et al. 2001). The occurrence of black stripe is correlated with leaf fall, and it is often categorised as the second phase of the leaf fall disease. Spores of the pathogen are spread by rain splash from the infected leaves to the tapping panel (Johnston 1989). Experiments in Sri Lanka showed that, under field conditions, a tapping knife did not transmit black stripe and that naturally infected trees showed a high incidence of panel infection close to ground level (Liyanage et al. 1984). Later experiments indicated that *Phytophthora meadii* was isolated from soil in a rubber plantation during epidemics of pod and leaf diseases (Liyanage and Wheeler 1991). Stem or patch canker, another phytophthora disease on rubber, is also associated with the occurrence of black stripe disease, leaf fall and pod rot. Stem or patch canker is common in rubber-growing countries but recent economical losses are relatively minor. Stem canker has been reported in countries in Southeast Asia including Malaysia (Chee 1971), Myanmar (Johnston 1989), and India (Mondal et al. 1994).

Extensive surveys of rubber diseases caused by *Phytophthora* in Thailand have been made since 1976. In general, leaf fall and black stripe are estimated to affect around 10% of the total growing area. An early record of severe damage from leaf fall and black stripe diseases was in 1976 (Kajornchaiyakol 1977). Leaf fall and black stripe outbreaks occurred on the east and the southwest coasts, of Thailand including Chuntaburi, Trad, Ranong, Phanga, Krabi, Phuket, Trang and Satun provinces (Figure 6.5.1 and Table 6.5.1), which cover about 100,000 ha. In the susceptible clone RRIM600, leaf fall occurred in 90–100% of the trees, which led to a 40% drop in yield (Kajornchaiyakol 1977). In 1979, although the area affected was reduced to 2000 ha, the disease severity was similar to that recorded in 1976 (Kajornchaiyakol 1980). A later survey indicated that damage by phytophthora diseases was reduced in southwest Thailand (Chantarapratin et al. 2001) due to replanting with rubber clones that are more resistant to *Phytophthora*.

Disease Symptoms

Phytophthora infection on rubber often begins on young pods and causes pod rot. The infected pods turn black and remain on the tree, dried up and unopened. After pod rot, the infection spreads to leaves and causes leaf fall (Figure 6.5.2). Infected leaves fall in large numbers, forming a carpet on the ground. Leaf blades of shed leaves show few signs of infection (Figure 6.5.3). A typical symptom of phytophthora leaf fall is the appearance of dark-brown lesions on the petioles with one or two drops of coagulated latex in the centre of the lesion (Figure 6.5.4). The lesion is often found near the base of the petiole and causes the premature abscission of the leaf. However, the lesions can occur anywhere along the length of the petioles. Heavy defoliation may lead to dieback of terminal branches (Chee 1968; Runner 1969; Johnston 1989).



Figure 6.5.1 Rubber trees showing the effects of an outbreak of leaf fall and black stripe disease in Thailand.

Table 6.5.1 Distribution and severity of *Phytophthora* leaf fall disease on rubber in Thailand.

Provinces	Location	Infested area (ha)	Leaf drop (%)
Trad	East	32	3
Chumporn	Southwestern coast	2	10–75
Songkhla	Southwestern coast	5,280	80
Phangnga	Southwestern coast	9,197	80
Krabi	Southwestern coast	5,596	100
Trang	Southwestern coast	76,800	100
Satun	Southwestern coast	560	80

Source: Kajornchaiyakol (1977).

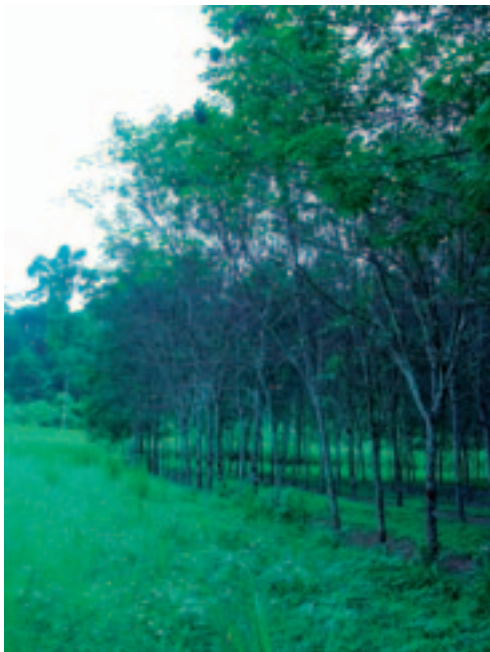


Figure 6.5.2 Leaf fall from rubber trees, caused by *Phytophthora* infection.



Figure 6.5.3 Leaf blades from *Phytophthora*-infected rubber trees.

The rubber panel is continually injured in the tapping process, so it is vulnerable to fungal infection. *Phytophthora* attacks the tapping panel and causes black stripe disease. Symptoms of black stripe at the early stage of infection appear as a slightly discoloured area above the tapping cut. Later vertical depressions occur on the renewing bark (Figure 6.5.5). When the bark is removed, dark lines are visible, corresponding to the depressions on the panel surface (Figure 6.5.6). As the infection progresses, the black lines extend internally into the wood, coalesce forming broad lesions (Figure 6.5.7) and finally spread the full width of the panel. The infection also causes uneven regeneration of the panel bark. In susceptible clones, protuberance may be formed (Figure 6.5.7). This makes it difficult to tap again (Johnston 1989).

Occasionally, infection occurs on untapped bark and induces stem canker. Symptoms of stem canker begin with discolouration of the bark. This is followed by latex exudation (Figure 6.5.8). A dark-purplish liquid oozes from the damaged bark, forming a coagulum with a distinct odour, and which often causes the bark to bulge and split open (Figure 6.5.8). Internally, the disease symptoms are similar to black stripe disease but occur on the stem, mature branches and/or the branch-stem intersection (Pereira et al. 1995). When the disease occurs at the base of the trunk, it is called patch canker. In comparison, stem canker is less important than black stripe disease in terms of disease incidence.



Figure 6.5.4 Dark-brown lesions on the petioles with one or two drops of coagulated latex in the centre of the lesion are a typical symptom of phytophthora leaf fall

Pathogens

Several species of *Phytophthora* have been reported to be responsible for diseases in rubber, including *P. botryosa* (Chee), *P. capsici* (Leonian), *P. citrophthora* (Smith and Smith) Leon, *P. meadii* McRae,

P. nicotianae Breda de Haan, and *P. palmivora* (Butl.) Butl. However, the most common *Phytophthora* species causing disease in rubber are *P. palmivora*, *P. meadii*, and *P. botryosa* (Table 6.5.2). In Brazil, *P. capsici* was reported to be the main species associated with black stripe and stem canker, but *P. palmivora* and *P. citrophthora* were also isolated from diseased rubber (Dos Santos et al. 1995). The predominant *Phytophthora* species infecting rubber in India, Myanmar, and Sri Lanka is *P. meadii* (Liyanage

1982; Kochuthresiamma et al. 1988; Johnston 1989), whereas in Malaysia, Thailand and Vietnam *P. palmivora* and *P. botryosa* are implicated (Chee 1969, 1971; Tsao et al. 1975; Duong et al. 1988). In China, although the main species involved appears to be *P. citrophthora*, other species including *P. palmivora*, *P. meadii*, *P. nicotianae* and *P. capsici* were also found to infect rubber (Zeng and Ward 1998). *P. citrophthora* was reported for the first time infecting rubber in Indonesia in 1989 (Liyanage and Wheeler 1989).



Figure 6.5.5 Symptoms of black stripe disease, caused by *Phytophthora* on the tapping panel of a rubber tree.



Figure 6.5.7 Lesions extending from the bark into the wood.



Figure 6.5.6 Under-bark depressions.



Figure 6.5.8 Latex exudation from a stem canker.

Disease Control

Control measures for phytophthora diseases on rubber involve fungicide application, planting of tolerant clones, using appropriate cultural practices, and disease forecasting. Copper oxychloride in mineral oil is extensively used in India, Malaysia and Sri Lanka as a preventive spray in the management of phytophthora leaf fall (Jayasinghe and Jayaratne 1996). However, application of chemicals to control leaf fall from mature rubber trees is impractical and costly due to the height of the trees and the large plot sizes. Metalaxyl, oxadixyl, catafol, folpet or mancozeb are recommended for panel treatment to control black stripe (Tan 1983; Jayatissa et al. 1994; Jacob et al. 1995). In India, 0.8% phosphorous acid gave effective and economic protection of tapping panels of the rubber trees from black stripe disease when applied at weekly intervals (Jacob et al. 1995).

Chemical control alone is increasingly becoming an unacceptable strategy due to the impact on the environment. Steps have already been taken to introduce an integrated approach to phytophthora disease management on rubber, with special emphasis on genetic resistance (Radziah and Hashim 1990; Jayasinghe and Jayaratne 1997). Screening and genetic improvement of rubber for resistance to *Phytophthora* have been implemented in

Southeast Asia (Pattanakul et al. 1975; Pillai et al. 1989; Jayasinghe and Jayaratne 1996). Several tolerant clones have been established and successfully planted in areas where the diseases are endemic. Tolerant clones recommended for Southeast Asia include RRIM712, PR255, PR261 and GT 1 (Anon. 1986). Previously popular rubber clones RRIM600 and PR 107 have been found to be susceptible to phytophthora diseases in most countries in Southeast Asia (Johnston 1989).

Forecasting phytophthora epidemics on the basis of weather data is saving unnecessary fungicide applications. Since rainfall coincides with the presence of pod rot in the field, which gives rise to phytophthora leaf fall and is subsequently followed by black stripe, fungicide should be applied with the onset of the leaf fall and continued for 2–4 weeks after the rain has ceased (Satchuthananthavale and Dantanarayana 1973).

Cultural practices also pay an important role in phytophthora disease management. In Thailand, weed control in rubber plantations is recommended as a means of suppressing disease development by reducing humidity during the long periods of rainfall (Pattanakul et al. 2001). In addition, experiments conducted in Malaysia indicated that factors leading to black stripe disease were the tapping of wet rubber trees during pod infection

Table 6.5.2 *Phytophthora* species associated with rubber diseases.

Species	Country	Reference
<i>P. botryosa</i>	Malaysia Thailand Vietnam	Chee (1968) Tsao et al. (1975) Duong et al. (1998)
<i>P. capsici</i>	Brazil China	Dos Santos et al. (1995) Pereira et al. (1995) Zeng and Ward (1998)
<i>P. citrophthora</i>	Brazil China Indonesia	Dos Santos et al. (1995) Zeng and Ward (1998) Liyanage and Wheeler (1989)
<i>P. meadii</i>	India Myanmar Sri Lanka	Kochuthresiamma et al. (1988) Johnston (1989) Liyanage (1982) Jayatissa et al. (1994)
<i>P. palmivora</i>	Brazil China Indonesia Malaysia Sri Lanka Thailand Vietnam	Dos Santos et al. (1995) Pereira et al. (1995) Zeng and Ward (1998) Parnata (1983) Chee (1969) Dantanarayana et al. (1984) Tsao et al. (1975) Duong et al. (1998)
<i>P. nicotianae</i>	China	Zeng and Ward (1998)

(Peries 1976). Also, it has been found that phytophthora disease intensity increased at tree-planting densities above 500 per ha (Anon. 1973).

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6.6 Phytophthora Diseases of Durian, and Durian-Decline Syndrome in Northern Queensland, Australia

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Abstract

Durian is the most popular fruit in Southeast Asia, with high economic and cultural value to the producing countries, which include Indonesia, Malaysia, Philippines, Thailand and Vietnam. The greatest threat to durian production in all countries is *Phytophthora palmivora*, which affects all stages of the cropping cycle. This chapter describes the diseases caused by *P. palmivora*, and their epidemiology. The chapter also describes a perplexing durian-decline syndrome which occurs in northern Queensland, where it appears that *P. palmivora* is operating in a complex with *Pythium vexans* and nematodes from the *Xiphinema* genus. Early control recommendations and their limitations are described, which leads to a discussion of integrated disease management principles and their applicability to the control of phytophthora diseases in durian.

The high-rainfall conditions under which durian is grown are conducive to the development of phytophthora diseases. In Southeast Asia, the most serious diseases of durian are caused by *Phytophthora palmivora*. *Phytophthora palmivora* causes seedling dieback, leaf blight, root rot, trunk cankers, and pre- and postharvest fruit rots (Lim 1997). *Phytophthora nicotianae* has also been reported as being a causal agent of durian root rot and canker on a few occasions in Malaysia (Bong 1993). Postharvest fruit rots result in 10–25% losses of durian fruits (Lim 1990).

Phytophthora Diseases in Durian

The genus *Phytophthora* is considered to be one of the most important plant pathogens worldwide. It

has been identified as a major impediment to the development of a sustainable durian industry in Australia (Zappala 2002). *Phytophthora nicotianae*, *P. botryosa* and *P. spp* (durian) have been identified as pathogens of durian (Bong 1993; Erwin and Ribeiro 1996; Brown 1997; M. Weinert, pers. comm.), but the most destructive and economically significant diseases are caused by *P. palmivora* (Navaratnam 1966; Pongpisutta and Sangchote 1994; Lim 1998). *Phytophthora palmivora* is endemic to Southeast Asia, where there is much genetic diversity, and balanced populations of the A1 and A2 mating types occur (Lee et al. 1994; Mchau and Coffey 1994) To date only the A1 mating strain has been associated with diseases in durian (Lim 1990; Lee et al. 1994).

Although essentially a soil-borne pathogen, *P. palmivora* is adapted to attack aerial parts of the plant (Chapter 3.1) and, as a result, can affect all organs of durian and all stages of the cropping cycle. The most devastating diseases include seedling dieback, foliar blight, patch canker of the trunk and branches, and pre- and postharvest fruit rots (Lim 1990).

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Seedling dieback and foliar blight

Seedling dieback is common in durian nurseries and, where disease management is poor, losses can be as high as 50% (Lim 1990). Infection is commonly initiated at the young stem, or at the graft union in double rootstocks, with a conspicuous lesion. Under suitable conditions the infection quickly spreads to the roots and leaves, producing dieback symptoms. When the root system becomes extensively rotted, and/or the main stem is girdled, the seedling will die.

Leaf blight may occur on individual leaves or, in extreme cases, the whole foliage may become diseased (Figure 6.6.1), killing the seedling from the top (Lim 1990). Although more common in nurseries, foliar blight can occur also in orchards under conditions of extremely high disease pressure. By the time foliar symptoms become apparent in an orchard, infections in other organs of the tree are generally well advanced and remediation is difficult if not impossible (Bong 1993).



Figure 6.6.1 Seedling blight of durian caused by *Phytophthora palmivora*.

Patch canker of the trunk and branches

Patch canker may begin at the soil line or at the crotch region (Lim 1990), although in Thailand cankers are often first observed on branches high in the tree canopy (S. Sangchote, pers. comm.). Cankers first become evident as discrete wet-looking patches on the bark. The patches eventually coalesce to produce a conspicuous canker that exudes a reddish/brown resinous substance. When the bark is removed, a reddish/brown lesion is revealed in the cortex which, in a healthy state, is cream to pink (Figure 6.6.2). Infection commonly extends into the xylem and, when the main trunk or root is girdled, leaves wilt and become chlorotic and branches desiccate, producing classical dieback symptoms. Lesions may also be found on feeder and large lateral roots (Bong 1993), in which case root rot will contribute to the above-ground symptoms. Infected trees may survive many years from the time of initial infection, as pathogen activity slows considerably during the dry season, although the stress of drought on the host may speed up infection in the following rainy season (Cook 1975; Lim 1990).



Figure 6.6.2 Lesion beneath the bark at the lower trunk of a durian tree. The lesioned tissue is brown compared to the creamy/pink colour of the healthy tissues.

Pre- and postharvest fruit rot

The incidence of preharvest fruit rot due to *P. palmivora* in Malaysian durian orchards can be as high as 30%, depending on the weather and microclimate (Lim 1990; Lee 1992). The following disease description is from Lee et al. (1994) and

applies to pre- and postharvest diseases (see also Figure 6.6.3):

The disease first appears as tiny water-soaked lesions on the outer skin which later coalesce to form dark to black brown regions. White powdery masses of sporangia form on the lesion surface, especially when conditions are moist and humid.

The rot spreads rapidly through the skin and pulp to the seed, making the fruit unmarketable and inedible (Lim 1990; Lee et al. 1994).

P. palmivora can infect fruit at all stages of development, and preharvest infections can result in postharvest rots (Johnson and Sangchote 1994). Preharvest infection may not be apparent at the time of harvest, or infection can occur during harvest when fruit is allowed to come into contact with infested orchard soils. In either case, if conditions are favourable during transit, *P. palmivora* can spread throughout, and ruin whole consignments of fruit. Favourable conditions for postharvest infection of non-wounded fruit include high humidity (at least 98% relative humidity) for at least 72 hours (Chapter 3.2).



Figure 6.6.3 Durian fruit with large brown lesion caused by *Phytophthora palmivora*. Sporangia have formed in white powdery masses between the spines.

Disease Epidemiology

The most important characteristics of *P. palmivora*, from an epidemiological perspective, are short generation time, great reproductive capacity under favourable conditions, and the production of deciduous sporangia that readily release zoospores in the presence of free water (Erwin and Ribeiro

1996). We have a good understanding of the epidemiology of *P. palmivora* in cocoa (Chapter 4.1).

P. palmivora is endemic to tropical Southeast Asia and survives in soil and on abscised or thinned durian fruit that has been left on the orchard floor (Lee 1992; Chapter 3.1). Disease develops in durian nurseries where humidity is consistently high due to a high density of seedlings, excessive watering (sometimes with infested water), excessive shade, inadequate ventilation and poor drainage. The situation is exacerbated by the maintenance of seedlings at ground level where they are exposed to soil-splash of infested water (Figure 6.6.4). The deciduous sporangia produced on the surface of stem or foliar lesions are spread by seedling-to-seedling contact, irrigation and human activities. Potential infection courts include wounds or stomata, which are prevalent on leaves, petiole and young stems (Chapter 3.2).



Figure 6.6.4 Durian seedlings maintained in a nursery on bare soil at ground level. Water has ponded around the plants and the seedlings are subject to splash of soil and water infested with *Phytophthora palmivora*.

Of particular concern is the practice in some nurseries of using phosphonate as a soil drench, because although it will suppress disease development in the plant, the pathogen remains viable, and its presence is merely masked. In this way, infested soil is unwittingly introduced into orchards.

Conditions that encourage high humidity in the orchard exacerbate disease. These include close plantings with intertwining dense canopies (Figure 6.6.5), poor drainage (Figure 6.6.6), poor hygiene

(Figure 6.6.6) and cultivation of susceptible varieties (Erwin and Ribeiro 1996; Lim 1990).

Evidence from research in Papua New Guinea indicates that beetles are key agents in the transmission and spread of *P. palmivora* in cocoa (Konam 1999; Konam and Guest 2004; Chapter 6.2). Durian patch cankers are attractive to boring beetles (Cook 1975) and it is likely that some of the many insects that occur in durian orchards (Figure 6.6.8) act as vectors of the abundant deciduous sporangia that form on infected organs, particularly fruit. Tent-building ants and termites also carry infested soil up the tree. The transmission of sporangia by insects may explain the initiation of infections high in the canopy, as observed in Thailand.

Durian fruit generally ripens in the early rainy season when climatic conditions for infection and colonisation of the host are optimal. The pathogen

can penetrate the cuticle of the fruit in the region between the spines, or invade through wounds or stomata (Chapter 3.2). Abundant sporangia are produced on the developing lesions (Figure 6.6.3), and the wind and rain associated with the monsoon facilitate both wounding and the dissemination of sporangia within the already infected tree and throughout the orchard. Drops of rain carrying sporangia collect at the stylar end of the fruit, causing infection that spreads upwards on the fruit in a concentric pattern (Lee et al. 1994), and water dropping from the fruit carries sporangia to fruit and branches below. Infected fruit or leaves drop prematurely, returning inoculum to the soil. Failure to remove infected fruits will provide an energy source for an explosive increase of inoculum. Cryptic infections on ripe fruit will initiate postharvest rots during transit and storage.



Figure 6.6.5 Dense plantings and closed canopies lead to high humidity in the orchard providing ideal conditions for the proliferation of *Phytophthora palmivora* and infection of durian. Note the high watertable.



Figure 6.6.6 In some durian growing regions of Vietnam 'moats' are created around trees to facilitate manual irrigation (water is pumped into the moat in the dry season). However, water is trapped against the trunk of the tree in the wet season causing disease.



Figure 6.6.6 *Phytophthora*-infected durian fruit in an irrigation channel where they will produce inoculum for further infections within the orchard.

Disease Control Options — a Historical Perspective

An understanding of the epidemiology of the moisture-loving *Phytophthora* led to recommendations for cultural disease control as early as the 1960s; they include good drainage and methods to improve ventilation and reduce humidity, such as wider spacing of trees, pruning of lower branches and the removal of weeds from under the canopy (Navaratnam 1966; Cook 1975).

Durian cultivars have historically been selected for fruit quality and productivity. Disease resistance was a secondary concern and reports of it anecdotal until 1971, when the first screening studies were conducted in Malaysia (Lim 1998). An underutilised source of resistance potentially exists in wild and semi-wild populations of *Durio* spp. and closely allied genera growing in Malaysia and Indonesia, the centre of diversity (Lim 1998). Techniques developed to identify disease resistance characteristics in durian are discussed in Chapter 8.4. Once identified, resistance can be exploited through plant-breeding programs, although both require a long-term commitment of funds and time. An alternative and

more rapid method of producing disease-resistant planting material is to use the resistant cultivar as a rootstock, onto which a scion with desirable commercial qualities is grafted (Lim 1998). This method is practised in Thailand where farmers routinely use Chanee as a rootstock due to a perceived disease-tolerance relative to other cultivars.

Recommendations for the chemical control of patch canker in durian did not change greatly between 1934, when the disease was first reported, and the mid 1990s (Lim 1990; Erwin and Ribeiro 1996). The main control option was the removal of the cankered tissue and painting the wound with an antimicrobial chemical and, in some cases, covering it with a dressing or tar (Cook 1975; Lim 1990; Lee 1992; Bong 1993; Erwin and Ribeiro 1996). This method gave inconsistent results, probably as there is limited penetration of the chemical into woody tissues and the fungicide is easily washed away. In addition, the process is laborious and expensive, and there were varying levels of diligence in reapplication (Lee et al. 1994).

The choice and effectiveness of fungicides to treat phytophthora diseases has increased over the years. The use of basic disinfectants gave way to protectants, including improved copper



Figure 6.6.8b A millipede moving over a weeping canker on the trunk of a durian tree, with the potential to pick up infectious propagules for distribution elsewhere in the orchard or further up the tree.

Figure 6.6.8a Termites build mounds around durian trunks with *Phytophthora*-infested soil increasing the risk of trunk canker.

formulations, dithiocarbamates (e.g. mancozeb) and phthalimides (e.g. captafol), followed by systemic fungicides effective against oomycetes, such as the acylalanines (e.g. metalaxyl) and the phosphonates (e.g. fosetyl-al, phosphorous acid) (Navaratnam 1966; Lim 1990; Kendrick 1992).

New formulations with different modes of action brought alternative recommendations for the methods of application. These included soil drench, foliar spray and, most recently, for woody perennials, direct injection into the trunk with the systemic formulations (Lim 1990). Some systemics, including metalaxyl, act on specific biochemical targets within the fungus, so it wasn't long before resistance to the fungicide was reported in *P. infestans* (Davidse et al. 1981; Kendrick 1992; Fungicide Resistance Action Committee (FRAC) website at <www.frac.info/publications/FRACCODE_sept2002.pdf>). New reports of fungicide resistance in other species of *Phytophthora*, and in *Pythium*, continue to mount (Parra and Ristiano 2001; Taylor et al. 2002). To reduce the risk of fungicide resistance in *P. palmivora*, a combination of protectant fungicides and metalaxyl is recommended for topical application (Lim 1990; Bong 1993).

Durian fruit rot was controlled by spraying with the same formulations recommended for patch canker and other diseases. However, there were unresolved issues about residues, stains on the skin left by the chemicals, and the difficulty of reaching fruit in the upper canopy without the aid of expensive high-pressure equipment (Lim 1990; Lee et al. 1994).

In the late 1970s, phosphonate emerged as a chemically simple, relatively inexpensive, yet highly effective weapon against *P. cinnamomi* diseases in avocado. Due to its systemic nature and ambimobility it was particularly suited to application as a trunk injection (Darvas et al. 1984), which circumvented the problem of fungicide wash-off. By the late 1980s, phosphonate trunk-injection was being successfully applied in other *Phytophthora* pathosystems, including *P. palmivora* on cocoa (Guest et al. 1994) and durian (Lim 1990; Lee et al. 1994) although phytotoxicity was reported in durian when rates of phosphonate application exceeded 25 g active ingredient (a.i.)/year (Lee 1992).

A common theme in disease control recommendations is the importance of early treatment, and the difficulty of saving trees that are suffering several phytophthora diseases simultaneously (Bong 1993; Erwin and Ribeiro 1996). Initial inoculum level is the key element in

Vanderplank's model for epidemics in multi-cyclic pathogens such as *P. palmivora* (Erwin and Ribeiro 1996). Erwin and Ribeiro (1996) make the following points:

- inoculum can be reduced but not entirely eliminated through scrupulous hygiene
- the pathogen is less likely to sporulate on planting material with vertical resistance, but vertical resistance is elusive (especially in woody perennials like durian), and usually not durable because of the reliance on a single gene, which puts great selection pressure on the pathogen to adapt
- a chemical blitz can potentially reduce the inoculum levels to zero, but eradicans such as methyl bromide are being phased-out due to the environmental hazards they pose and, as already mentioned, *Phytophthora* is showing tolerance to some of the most-effective selective fungicides currently available.

In highlighting the fact that no single method will effectively and sustainably reduce inoculum levels and thus control multi-cyclic pathogens, Erwin and Ribeiro (1996) succinctly present the case for integrated disease management. The case for integrated disease management is bolstered by a rise in our consciousness of environmental and health issues, which makes our past reliance on chemicals for disease control unacceptable.

Integrated Disease Management

Integrated disease management (IDM) is the long-term control of crop diseases to economically acceptable levels through a holistic approach which combines:

- the use of resistant varieties where available
- cultural control methods
- biological control methods
- the judicious application of appropriate chemicals.

Durian is an ideal model for the development of IDM strategies because the high value of the fruit provides impetus for the intensive and continuous orchard management practices required in a perennial tree crop.

The principle of integrated management of phytophthora diseases in durian has been promoted since the early 1990s (Lim 1990; Bong 1993; Lee et al. 1994) but, for the most part, detailed recommendations were lacking or implementation patchy. A systematic approach to developing recommendations was undertaken as part of an

ACIAR-funded project 'Management of *Phytophthora* diseases in durian' (Project No. PHT/1995/134), which commenced in 1998. As part of the project, practical disease-control options were investigated, regionally optimised and disseminated to durian farmers in Thailand, Vietnam and Australia. The project culminated in a workshop that was held in Chiang Mai, Thailand in November 2002. The presentations there formed the nucleus for the production of this monograph.

The recent, rapid expansion of the durian industries in Thailand and Vietnam has seen the establishment of orchards on marginal sites, including rice paddy in Vietnam (Figure 6.6.9), where phytophthora diseases can be exacerbated. Major issues facing the durian industries in Thailand and Vietnam and investigated as part of Project PHT/1995/134 included:

- the need to identify sources of disease resistance in durian and the development of tolerant rootstocks (Chapter 8.2)
- poor practice in durian nurseries resulting in the release of infected planting material (Chapters 7.1 and 8.3)
- an incomplete understanding of the epidemiology of *P. palmivora* in durian, which hampers effective management (Chapters 3.1 and 2.2)
- an incomplete understanding of the effect of current management practices on disease incidence and development (Chapter 7.2 and 8.3)
- the lack of specific recommendations for the rate and timing of phosphonate trunk-injection to ensure efficient application and effective disease control (Chapter 6.3 and 8.4).

Durian-Decline Syndrome in Australia

Although the fledgling durian industry in Australia is facing many of the same issues as Thailand and Vietnam, the major problem in northernmost growing areas in Queensland is a devastating decline syndrome. Durian-decline syndrome (DDS) involves the rapid dieback of branches, necrosis in the cortex of feeder roots and eventually tree death (Figure 6.6.10). The symptoms are initially suggestive of disease caused by *P. palmivora*, except that cankers are rare and trees do not respond to trunk-injection with phosphonate. In an attempt to determine the cause of DDS, 13 affected farms were surveyed in a dry season (July–September 2001) and the following wet season (February–April 2002).

P. palmivora was isolated from the roots of affected trees on 12 of the 13 farms in the dry season, and all farms in the wet season. *Pythium vexans* de Bary was recovered from the roots of diseased trees on all 13 farms in both seasons. *Pythium vexans* was isolated from 68% of diseased trees, while *P. palmivora* was isolated from 24% of diseased trees in the dry season. In the wet season *P. vexans* was isolated from 45% of diseased trees, while *P. palmivora* was isolated from 35% of diseased trees. *Xiphinema* sp., a root-hair-feeding, plant-parasitic nematode, was also recovered from 12% of trees sampled. These results suggest a possible synergism between *P. palmivora*, *P. vexans* and plant-parasitic nematodes as the complex cause of DDS in northern Queensland.

The pathogenicity of *P. palmivora*, *Pythium vexans*, or a combination of the two pathogens, was tested on 3-month-old durian seedlings cv. Monthong. Inoculum of *P. palmivora* (chlamydospores) and *P. vexans* (oospores) was prepared using the submerged culture method described by Tsao (1971). A spore suspension (approximately 1×10^5 spores) was applied to the potting medium in each pot. Four replicate plants were used per treatment. An uninfected treatment was included for comparison. Two weeks after the inoculum was



Figure 6.6.9 The establishment of a new durian orchard in a rice paddy in the Mekong Delta region of Vietnam. The mounds on which the seedlings are planted, are expanded each year to accommodate the lateral growth of the root system. Eventually there will no longer be room to plant the rice.



Figure 6.6.10 Advanced symptoms of durian decline syndrome in far-north Queensland, Australia.

applied, the pots were placed in plastic trays and filled with water to a depth of 25 mm to saturate the soil by capillary action, which stimulates chlamydospore and oospore germination, sporangial development and zoospore release.

After 3 days, the pots were removed from the trays and the soil allowed to drain. Thereafter, plants were hand-watered as required. Plant roots were assessed for root rot after a further 6 weeks. Disease-affected roots were plated onto selective culture media and *P. palmivora* and *P. vexans* were re-isolated from infested plants.

Plants inoculated with *P. palmivora* showed obvious rotting of, and a reduced number of, feeder roots. Feeder roots of plants inoculated with *P. vexans* appeared necrotic compared with controls but there was no obvious reduction in the number of roots. *P. vexans* may cause a reduction in the efficiency of affected feeder roots. A combination of *P. palmivora* and *P. vexans* failed to increase the severity of root rot compared with *P. palmivora*, which may have been a function of insufficient time under waterlogged conditions. Further experiments, including nematodes, are warranted.

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7

Managing Phytophthora Diseases



7.1 Principles of Phytophthora Disease Management

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Abstract

In order to limit the incidence and severity of diseases caused by *Phytophthora*, effective management strategies are needed. Management of phytophthora diseases is based on a number of principles such as avoiding infection through basic hygiene, limiting susceptibility through drainage and irrigation, improving soil health, use of disease-resistant germplasm, and biological and chemical control. Although the components are discussed here in a sequential order, effective control of phytophthora diseases is often only achieved through the integrated use of a number of these strategies.

Introduction

There are more than 60 described species of *Phytophthora* and all known species are plant pathogens. Each species can cause disease in from a few to over a 1000 different plant species. Hence, there are a few thousand diseases in a wide range of plants caused by the various species within the genus *Phytophthora*. Each of these diseases will have its own characteristics, which makes it difficult to generalise disease-control methods. However, it is important to understand the most common contributing factors that underpin the control of phytophthora diseases. Only an in-depth understanding of these underlying factors, coupled with a detailed understanding of the agronomics of the crop will allow one to develop effective, integrated disease control methods. The aim of this chapter is to provide an underlying basis for disease control by discussing a wide range of management practices available under the following headings: (1) cultural practices, (2) resistance breeding, (3) biological control, (4) fungicides, and (5) phosphonates.

Cultural practices

The effectiveness of control strategies depends on the ability of an individual species of *Phytophthora* to survive, either as a saprophyte or as dormant spores. Generally, mycelium and zoospores survive for only a few weeks, while chlamydospores may survive for 6 years, and oospores for 13 years (Erwin and Ribeiro 1996). Some species, however, such as *P. cinnamomi*, appear to have a high saprophytic ability (Zentmyer 1980) while others such as *P. palmivora* do not (Ko 1971).

Quarantine, nursery and orchard hygiene

Quarantine is the only means of preventing the introduction of a new pathogen into an area. Quarantine is also extremely important in nurseries where millions of plants are produced each year, providing opportunities for the rapid spread of *Phytophthora*. In areas where *Phytophthora* has not been recorded, exclusion is essential. Exclude animals by fencing, minimise the movement of vehicles and people through the orchard, remove soil from vehicles, boots and tools before they are brought into the orchard, plant only disease-free and resistant trees, and divert water run-off from adjacent orchards (Broadley 1992).

In nurseries, potting mixes should be steamed to kill *Phytophthora* inoculum, and only certified *Phytophthora*-free planting material should be used (Chapter 7.2). Good hygiene in orchards is a

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fundamental component of effective pest management. It is virtually impossible to eradicate *Phytophthora* from the soil. Therefore, if *Phytophthora* is present, metalaxyl should be used to minimise disease development. Roadways, interrows and equipment should be kept clean. The site should be well-drained to avoid water from forming ponds that may subsequently allow *Phytophthora* to proliferate. Orchards should also be kept free of rotting plant debris that may be infected with *Phytophthora* (Broadley 1992).

Drainage and irrigation

Excess irrigation and rainfall are considered to be the most important factors that increase the severity and spread of *Phytophthora*-incited diseases. In turn, the duration of free water, in soil or on foliage or fruit is the most important environmental factor in the development of disease caused by *Phytophthora* because it is during this time that propagules proliferate and infect (Erwin and Ribeiro 1996). In addition, zoospores, cysts and chlamydospores travel in the soil in irrigation water, rainfall run-off and movement of soil. Orchards should be established on land that is well-drained and not subject to flooding. Therefore, sloping ground is preferable. Ideally, the soil should be drained to a depth of 1.5 metres. Mounding of the soil around the tree promotes good drainage (Broadley 1992). Row crops should be planted on raised beds to prevent free water from contacting the plants (Erwin and Ribeiro 1996). To reduce the rate and extent of build-up of inoculum, plants should be irrigated less frequently so that free water drains away (Lutz et al. 1989). In areas where rainfall is the main source of water, optimal horizontal and vertical drainage are necessary to prevent water-logging. Spraying water on the trunks of trees should be avoided as constantly wet bark may encourage the development of cankers.

Organic amendments and mulching

Mulching stimulates plant root growth, increases nutrient uptake, decreases evaporation from the soil, increases soil-water holding capacity, reduces surface water run-off, facilitates drainage, regulates soil temperature, and provides a high level of nutrients for soil microbes (Aryantha et al. 2000). Amendments can either enhance or suppress disease, depending on their nature. *Phytophthora* is inhibited by alfalfa meal, cotton waste, soybean meal, wheat straw, chicken manure and urea. Ammonia and volatile organic acids released by decomposing organic matter kill *Phytophthora*, and the residual organic matter stimulates competitive

and antagonistic microorganisms in the soil (Lazarovits et al. 2001). While these mechanisms suppress the growth of *Phytophthora*, they may also create phytotoxicity to the plant roots, making them less attractive to colonisation by the pathogen (Erwin and Ribeiro 1996). Aryantha et al. 2000 showed that the addition of fresh or composted chicken manure to potting mix significantly reduced the survival of *P. cinnamomi* and the development of disease in lupin seedlings. Chicken manure more effectively suppressed *P. cinnamomi* and plant-disease symptoms than did cow or sheep manure. All composts increased soil organic matter, total biological activity, and populations of antagonistic actinomycetes, fluorescent pseudomonads, and fungi. However, chicken manure also stimulated the production of endospore-forming bacteria, which was positively correlated with lupin seedling survival. The addition of composted manures is necessary for disease development but it is not sufficient for biological control. Mulches may also reduce the impact of phytophthora root rot if used from the time of orchard establishment or if the disease is not too far advanced. The 'Ashburner system', based on improved drainage and mulches, has been successfully employed to manage phytophthora root rot of avocados (Broadley 1992). Chapter 7.3 reports that mulches are also effective in managing phytophthora root rot of papaya.

Companion and cover cropping

Companion cropping can reduce the impact of phytophthora diseases. For example, in the subtropics of Australia, banana and avocado are planted together. The bananas provide mulch and reduce soil water after heavy rain. This system reduces the impact of root rot caused by *P. cinnamomi*. Care must be taken to choose a companion crop that does not compete too heavily with the orchard crop. Cover crops, when incorporated into the soil, increase the amount of organic material, which encourages the growth of microbes that suppress *Phytophthora* (Broadley 1992).

Fertilisers

Some forms of nitrogen have been shown to favour an increase in disease, while other forms suppress disease (Schmitthenner and Canaday 1983). Generally, the role of fertilisers or nutrients in controlling or suppressing phytophthora diseases is unclear. Some reports indicate that fertilising improves plant vigour and hence resistance to disease, while others indicate that pathogen infection is favoured because of improved plant vigour (Erwin and Ribeiro 1996).

Suppressive soils

Soils that favour the expression of disease are conducive, while those that are inhospitable to plant pathogens are suppressive. The principal cause of suppressiveness is an increase in the population of antagonistic bacteria, fungi and actinomycetes. *Phytophthora*-suppressive soils have been reported in orchards and natural forests where, frequently, other soil-borne pathogens are also suppressed. Direct lysis of hyphae and inhibition of germination of chlamydospores of *P. cinnamomi* has been observed in suppressive soils. Suppression is attributed to the activities of soil-borne antagonists that may produce antibiotics active against *Phytophthora* (Halsall 1982). There are also a number of microorganisms which hyperparasitise oospores of *Phytophthora*.

Resistance

The success of resistance to *Phytophthora* in the field is determined by the interaction between the host, pathogen and the environment. Inoculum concentration and environmental conditions ultimately determine how effective host resistance will be in minimising disease. Generally, it is more difficult to find host resistance to pathogens that have a wide rather than a narrow host range. Resistance in the majority of hosts to different species of *Phytophthora* is non-specific in nature. However, a few species such as *P. fragariae*, *P. infestans*, *P. sojae* and *P. vignae* have gene-for-gene interactions with their hosts, and hence resistance is race-specific and frequently controlled by a single dominant gene in the host. Cultivar-specific resistance to *P. capsici* and *P. nicotianae* has been observed, and the mechanisms of resistance appear to be related to the physiology of the cultivars. There are three components of general resistance to *Phytophthora*: (i) resistance to penetration, (ii) restriction of growth of the fungus in the host, and (iii) reduced sporulation of the fungus on the host.

The use of resistant rootstocks to combat soil-borne diseases in perennial crops is a vital component of an integrated disease-management program. In avocado, resistance has been identified from *Persea americana* and some non-commercial relatives of avocado. However, under conditions that favour *P. cinnamomi*, such as soil waterlogging, good control is not achieved even with resistant or tolerant rootstocks (Erwin and Ribeiro 1996). A disadvantage of clonal rootstocks of avocado is that they can be more difficult and slower to establish than seedling rootstocks. Some rootstocks limit the rate of disease development by rapidly regenerating feeder roots;

in others, infection of the root is minimised due to natural resistance mechanisms (Broadley 1992). General resistance to *P. citrophthora* and *P. nicotianae* has been developed in many rootstocks onto which grafts of commercial citrus species can be made (Erwin and Ribeiro 1996).

Resistant rootstocks can be obtained from seedlings generated from selected resistant/tolerant cultivars or by using marcotted seedlings developed from selected cultivars. Marcotted seedlings have been used to produce disease-tolerant rootstocks of durian (Lim 1998). Using *Phytophthora*-resistant or tolerant rootstocks as planting material has the added advantage of producing uniform trees (Broadley 1992). In New Guinea, efforts aimed at identifying resistance to pod rot in cocoa have been largely unsuccessful, and cultural and chemical management strategies remain the most viable methods of control (Holderness 1992). Resistance to bud rot and nut fall caused by *P. palmivora* and *P. nicotianae* has been identified in coconut (Mangindaan et al. 1992).

Phytoalexins are antifungal compounds produced by plants in response to the invasion of a pathogen. These compounds are widely associated with host resistance. Phytoalexins are non-specific in their inhibitory action, and can be induced by physical and chemical treatments and by non-pathogens. Their production can be elicited in response to compounds commonly produced by pathogens, such as complex carbohydrates from fungal cell walls, and lipids, enzymes and polypeptides. Elicitation of phytoalexin production by *Phytophthora* infection has been demonstrated in a number of hosts (Erwin and Ribeiro 1996). The salicylic acid analogue, Bion (acibenzolar-S-methyl), activates systemic acquired resistance in plants and can increase resistance to *Phytophthora* (Ali et al. 2000).

Biological Control

Many of the experiments performed on biological control of *Phytophthora* have been centred on in vitro studies or pot trials and not field situations. Research on biological control has encompassed large-scale screening efforts without seeking further understanding of the interaction between biological control agents and *Phytophthora*. If disease management is to be heavily based on biological control, the research effort in this area will need to be significantly increased, as there are very few choices of biocontrol agents for *Phytophthora* or effective techniques to apply them. However, biological control does provide an attractive and

environmentally friendly option to control or suppress the development of phytophthora diseases.

Recent developments in biological control include the identification of biocontrol agents such as actinomycetes (You et al. 1996), and fungi including *Trichoderma* spp. (Chambers and Scott 1995), *Penicillium funiculosum* (Fang and Tsao 1995), *Gliocladium* spp. (Lim and Chan 1986; Heller and Theilerhedtrich 1994; Chambers and Scott 1995) and *Chaetomium globosum* (Heller and Theilerhedtrich 1994). These agents have all suppressed growth of *P. cinnamomi*, mostly by hyphal lysis, but can also promote the growth of the host (El-Tarabily et al. 1996). Numerous studies have examined biological control of *P. palmivora* in cocoa, using microbial antagonists such as *Bacillus* spp., *Aspergillus tamarii*, *A. gigentus*, *Botryodiplodia theobromae*, *Penicillium purpurescens* and *Pseudomonas fluorescens*, with some success (Galindo 1992). Two species of the soil-dwelling genus *Myrothecium* were found to reduce leaf rot caused by *P. palmivora* and *P. katsurae* in coconut. This fungal genus is found in both temperate and tropical soils, and hence provides a possible option for biocontrol of bud rot in coconut (Tuset et al. 1992).

Biological control activity can be manipulated by adding exotic antagonists to the soil, or by stimulating the activity of endogenous antagonists through the addition of mulches or composts (Erwin and Ribeiro 1996). For example, the use of organic media (mulches, composted pine bark etc.) that have high microbial activity and low pH (Hoitink and Fahy 1986; You and Sivasithamparam 1995), provide promising options to control *P. cinnamomi* in container-grown plants in nurseries. Organic amendments have also been successfully extrapolated to the field; for example, in the control of apple replant disease (Utkhede and Smith 1994). Mycorrhizae may also provide biological control against *P. cinnamomi* as identified in pines (Marais and Kotze 1976) and pineapple (Guillemin et al. 1994).

A range of endophytic fungi have been shown to protect cocoa against fungal pathogens, including *Phytophthora*. The primary mode of action of these endophytes appears to be through direct antagonism (Arnold et al. 2003). The possibility therefore exists to identify active endophytes and to inoculate seedlings at the nursery so that they are protected in the field.

Fungicides

Protectant

Bordeaux mixture

This is perhaps one of the oldest known fungicides, formulated in 1885 by Millardet to control the Oomycete *Plasmopara viticola*, which causes downy mildew on grapevine (Millardet 1885). Bordeaux mixture has been used to successfully control many diseases caused by different species of *Phytophthora*. The fungicide adheres well to foliage, but has a disadvantage in that its active ingredient, copper, can have a significant toxic affect in some plants and non-target organisms (Brown et al. 1998). In addition, Bordeaux mixture is a combination of copper sulphate and calcium hydroxide, and thus is somewhat labour-intensive to prepare and apply (Erwin and Ribeiro 1996). Also, in tropical areas with high rainfall, the fungicide may be washed off.

Systemic

Phenylamides (acylanilides)

This group of chemicals includes furalaxyl (Fongarid), metalaxyl (Ridomil) and benalaxyl (Galben). All three chemicals are active against the Peronosporales, but metalaxyl is the most widely used (Erwin and Ribeiro 1996). This fungicide is a xylem-translocated compound with an upward movement in plants in the transpiration stream (Edgington and Peterson 1977). Thus, metalaxyl and related acylanilide compounds have no effect on root diseases if applied as a foliar spray because they are not transported to the roots. Metalaxyl is usually applied as a soil drench and it is very effective (Guest et al. 1995). Due to its systemic nature, metalaxyl is transferred from seed, roots and leaves to new growth (Cohen and Coffey 1986) and is therefore effective at controlling infection beyond the roots. Metalaxyl is water soluble, and is effective against all species of *Phytophthora* in vitro at much lower doses than protectant fungicides. The biochemical mode of action of metalaxyl involves inhibition of RNA synthesis. It is highly inhibitory to sporangium formation, and also reduces chlamydospore and oospore formation (Cohen and Coffey 1986). It also has a high level of persistence within the plant. The presence of metalaxyl within the plant can prevent colonisation of leaf tissue by mycelium, because it inhibits the growth of hyphae (Erwin and Ribeiro 1996).

There are several disadvantages of using metalaxyl and related compounds: (i) root drenching is a wasteful method of fungicide application; (ii) chemicals are released into soil and water systems; (iii) soil microorganisms rapidly degrade metalaxyl,

reducing its persistence and effectiveness (Guest et al. 1995); and (iv) resistance has developed to it among populations of *Phytophthora*, particularly *P. infestans* (Cohen and Coffey 1986). The issue of metalaxyl-resistance has been partially addressed by application of metalaxyl in combination with a protectant fungicide, limited application of metalaxyl during a given growing season, and not using the fungicide for curative or eradication purposes (Erwin and Ribeiro 1996).

Phosphonates

This group of compounds is active against the Peronosporales. The term 'phosphonate' refers to the salts and esters of phosphoric acid that release the phosphonate anion in solution. Phosphonates are prepared by partially neutralising phosphorous acid (H_3PO_3) with potassium hydroxide. In this text, phosphonates will be referred to in a general context, and mention will also be made of a specific formulation of phosphonate, fosetyl-Al. Marketed under the name Aliette, this compound contains an aluminium salt of phosphonate (Cohen and Coffey 1986).

Phosphonates are xylem- and phloem-translocated (Ouimette and Coffey 1990), with both downward and upward movement in the host. They are non-persistent in the environment, as they are readily oxidised to phosphate by soil microbes, and they also have very low mammalian toxicity. The precise mode of action of phosphonates is unknown, but it is believed that they disrupt phosphorus metabolism in the pathogen, causing fungistasis and the consequent activation of the host defence responses (Guest et al. 1995).

The presence of phosphonate at concentrations below those required to inhibit mycelial growth *in vitro* disrupts the virulence of the pathogen, causing the release of stress metabolites that elicit host defences. The consequence is that many plant species treated with phosphonates respond to inoculation as though they were resistant. Hence, the effectiveness of phosphonates against plant diseases caused by Oomycetes depends on both the sensitivity of the pathogen to phosphonate and the capacity of the defence responses of the host. Therefore, there is a 'complex mode of action' in response to phosphonate treatment (Guest et al. 1995).

Because of the complex mode of action of phosphonates, results obtained from one host-cultivar combination cannot be extrapolated from results with analogous combinations. This is because of the great variation in sensitivity of

different isolates of a single *Phytophthora* species. In addition, phosphonate efficacy differs among host cultivars or species, perhaps due to differences in the type or extent of defence responses in the hosts (Guest et al. 1995). Although the fungistatic effect of phosphonates is not confined to the Oomycetes, it is inexplicably variable in its effect against some species of *Phytophthora*. For example, fosetyl-Al is active against tuber rot caused by *P. infestans*, but is not very effective in controlling the foliar phase of late blight of potato (Erwin and Ribeiro 1996), possibly indicating the activation of tissue-specific resistance mechanisms.

Because phosphonates are phloem-translocated, they can be applied to any part of the plant and theoretically be transported to all other plant parts according to source-sink relationships in the growing plant. Phosphonates spread rapidly throughout plant tissue; within a few minutes for small plants such as tomato, and within days for large trees such as avocado. Phosphonates can be applied either as a drench, foliar spray, stem-canker paint, or trunk injection for direct systemic control. Fungicides applied as foliar sprays and drenches are often limited in their effectiveness. This is because fungicide uptake into the plant tissue is generally poor, fungicide activity is rapidly lost due to degradation by soil and phylloplane microbes, and fungicides are lost to the environment through leaching and wash-off (Guest et al. 1995). Pressurised trunk injection forces the chemicals into the trees, minimising wastage and environmental contamination, and achieving maximum persistence (Darvas et al. 1984). For each host species and each disease, the injection rate, number of injection sites and the timing and frequency of injection need to be optimised. Although phosphonates persist very well in plant tissue, sequential applications are required to maintain concentrations essential to effective and durable disease control, especially in perennial crops.

Most of the hosts on which phytophthora diseases have been controlled by phosphonates are perennial fruit crops. Treatment is particularly effective because the fruits are strong metabolic sinks for the translocation of phosphonates, and because reduced disease in one season reduces the inoculum available in the following season. Trunk injection can be used to treat *Phytophthora* infections of roots, leaves, stems and fruits (Guest et al. 1995).

There do not seem to be many problems associated with phosphonate usage. Unlike metalaxyl, phosphonate-resistant isolates of *Phytophthora* have not been detected after more than 20 years of use.

Although some studies have shown that soil drenches of fosetyl-Al and phosphonates inhibit root growth and subsequent colonisation of the roots by mycorrhizal fungi, others have shown that application of fosetyl-Al enhances mycorrhizal colonisation (Guest et al. 1995). It is important to remember that phosphonates will not eradicate the pathogen or eliminate disease, but remain an excellent, cost-effective option for control of phytophthora diseases.

Conclusions

Effective disease control is rarely achieved through the application of a single disease-control method. In order to limit the risks associated with outbreaks of disease we need to use a number of different approaches in an integrated manner. Starting with disease-free planting material, site preparation and establishing good drainage will not only limit phytophthora disease severity but, also, the improved soil health will benefit the host plant directly. The planting of resistant material, if available, is a highly cost-effective way to control disease, but these trees will also benefit from improved drainage and good soil health. Chemicals can be used as a last option, as their use often involves a significant cash outlay for equipment and fungicides. The use of fungicides also requires knowledge about optimal timing of sprays, rates of application, additives and application methods, in order to be applied effectively. Throughout this monograph we have tried to give practical advice on how to integrate the different components of disease control in an effective manner to reduce losses due to *Phytophthora*.

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7.2 Nursery Practices and Orchard Management

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Abstract

Orchards are usually established using grafted planting material obtained from specialised nurseries. It is paramount that such planting material and accompanying potting mix is of high quality and free of disease. This chapter outlines the steps involved in the production of disease-free nursery stock. Healthy planting stock should also be planted in healthy soil, and the impact of fertiliser, water and canopy management options on disease are discussed. The Ashburner system, originally developed to manage *Phytophthora* in avocado orchards, is also outlined and its wider relevance to perennial horticulture is discussed.

Introduction

Plant disease epidemics are extremely rare in nature and when they do occur they are invariably associated with human activity. On farms and in orchards, plants are usually grown in monocultures with very limited genetic diversity, and are cultivated for maximum yield. The emphasis on growth rate, precocity and yield often imposes unnatural stresses on plants. Cultivated plants are propagated and transported to new regions or continents and immediately confront new environments and populations of pests, pathogens and other organisms. Conversely, the movement of plants sometimes introduces pests and pathogens as passengers into new environments, where they discover previously unknown hosts.

Phytophthora is a genus that has benefited from these agricultural and horticultural practices and has been the agent of several major plant disease epidemics in the last two centuries. To understand these epidemics and to develop management practices to manage the impact of these pathogens, it is essential to understand how the biology of *Phytophthora* enables it to successfully exploit agricultural and horticultural practices. The

development of successful management practices requires a thorough understanding of the life and disease cycles of *Phytophthora* species on each host plant in each environment. The aim of most disease management practices is to exclude or reduce the amount of primary inoculum and to reduce the rate of epidemic development by suppressing secondary inoculum.

Of all the disease management strategies available to farmers, the most fundamental is to use healthy planting material in a healthy soil under conditions that favour the growth and development of the plant. A wise investment of effort, time and money to establish a healthy orchard will lay the foundation for decades of sustainable production. Nursery practices that ensure disease-free planting material, thoughtful site preparation to encourage successful orchard establishment, and management practices based on a thorough appreciation of how to manage a sustainable orchard ecosystem will also minimise production costs, social costs and environmental damage.

Nursery Practices

All species of *Phytophthora* are at least to some extent soil-borne pathogens that are primarily dispersed in contaminated soil, water or, less commonly, in infected planting material. Therefore, nursery practices designed to prevent the dispersal of *Phytophthora* pathogens should focus on preventing

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the introduction and subsequent movement of infested soil and water.

The rapid expansion of the avocado industry in Australia in the 1970s created a shortage of planting material and exposed serious deficiencies in standard nursery practice that were directly responsible for the spread of dieback disease throughout the major avocado-growing regions of north-eastern New South Wales and south-eastern Queensland. As a result, a strict set of standards was developed through the establishment of a pioneering nursery accreditation scheme. Growers soon recognised that the extra cost of purchasing certified planting stock from a recognised nursery was compensated in a short period of time by the absence of dieback, lower disease-management costs, higher yield, higher quality and longer tree life. Nurseries recognised that their reputation was enhanced by supplying only certified, disease-free plants and that their extra costs were rewarded with price premiums. Those that chose not to invest in improving their practices quickly lost their business as growers purchased elsewhere.

The accreditation scheme is based on a sound understanding of the biology of the pathogen, and the role of soil and water in its dissemination (Pegg 1978). The key elements are:

- preventing the exposure of pots, plants, tools and irrigation hoses to contaminated soil by paving all walkways and surfaces and suppressing dust
- placing pots and containers on raised benches, preferably made from galvanised wire mesh
- sterilising all pots, containers, and tools, and storing them where there is no chance of contamination by soil or water
- using a soil-free or pasteurised growth medium
- regularly testing irrigation water
- regularly inspecting, roguing, containing and destroying diseased plants
- quarantining newly acquired propagating material
- restricting access to all nursery areas to prevent the introduction of contaminated soil or water
- training nursery workers in hygienic practices, including refraining from eating, drinking or smoking in the quarantine area.

Any soil or river-sand based potting mix, or substrates containing cocopeat, may potentially harbour *Phytophthora*. These substrates can be avoided, but as they are readily available and relatively inexpensive, they are the most common potting mixes used in many tropical countries. Alternatively, these substrates can be disinfested before use.

Pasteurisation is an effective technique that eradicates soil-borne inoculum in potting mixes, however it requires a significant capital expenditure for nursery operators. The potting mix is moistened to field capacity overnight, then heated to at least 60°C, but less than 82°C, for 30 minutes using a pressurised steam-air mixture. Solarisation, which involves heating moist potting mix to temperatures of 45–50°C at 20 cm depth under sheets of clear plastic, using the heat of the sun for a week or more, provides many of the growth benefits of both methyl bromide fumigation and pasteurisation if carefully monitored. Solarisation is a promising technique for tropical areas because of the low cost and technical requirements, but has the potential to generate a lot of waste plastic if the plastic sheets are of such low quality that they cannot be reused.

Another technique that eradicates pathogens from potting mix is anaerobic fermentation, using organic additives such as chicken manure, green silage and microbial supplements. Chicken manure releases ammonia and volatile acids, before stimulating the activity of antagonistic and hyperparasitic microbes, creating an actively suppressive soil ecosystem (Aryantha et al. 2000; Lazarovits et al. 2001). Methyl bromide fumigation is also effective but is no longer acceptable because of its adverse effects on human health and its role in the depletion of the ozone layer. Ultimately the safest, but most expensive, method is to use freely draining, soil-free potting mix, based on mineral substrates such as vermiculite, perlite or rice husks and composted hardwood bark. There is a great need to develop low-technology, low-cost, pathogen-free potting substrates for nurseries in Southeast Asia.

Orchard Establishment

There is little point purchasing disease-free planting material if the orchard soil is infested with the pathogen. Site selection is critical. A study of the previous cropping history will indicate the presence of soil-borne pathogens, and the threat these pathogens pose to the new crop. *Phytophthora* spp. thrive in soils with low organic-matter contents, low biological activity, and low water-holding-capacity soils that are prone to temporary ponding, and aerial dissemination is favoured in environments or microclimates with long periods of high relative humidity.

Once a site containing suitable soil has been identified and the orchard layout decided upon, drainage has to be attended to so that flooding and ponding is avoided, while appropriate irrigation is designed, if necessary. Planting on mounds or ridges

is widely practised and effective, especially in low-lying orchards or where the watertable is high. An extreme example is the transformation of rice paddies in the Mekong Delta to durian orchards by transplanting young trees on top of mounds in the paddy (Figure 7.2.1). Over successive seasons, the trees grow and the mounds are built wider until the canopy closes and rice is replaced. The flooding of rice paddies creates anaerobic soils, and eradicates *Phytophthora*, so that the planting site starts out free from the pathogen.

In orchards where the pathogen is known to exist from previous cropping experience or positive soil tests, the planting hole can be prepared to suppress or eradicate the pathogen from the root zone (Broadbent and Baker 1975). A practice common around Ba Ria-Vung Tau in Vietnam, where durian orchards are planted on old rubber plantations

where *Phytophthora palmivora* is present, is to dig holes approximately 50 cm deep and 100 cm in diameter, fill each hole with fresh chicken manure and green compost, cover with soil and compact (Figure 7.2.2). These develop into small silage pits that eradicate the pathogen over a period of 3–4 months, and also break any hardpans or impermeable laterite subsoils that may impede drainage. A small planting hole is excavated for the young tree. This practice will, however, only be effective in the long term if sufficient organic material is placed in the planting hole and a well-drained mound of sufficient height is created to allow effective drainage of water.

Where irrigation is necessary, methods that involve flooding bare ground, while convenient, should be avoided because they create 'swimming pools for zoospores' (Somsiri Sangchote, pers. comm.) (see

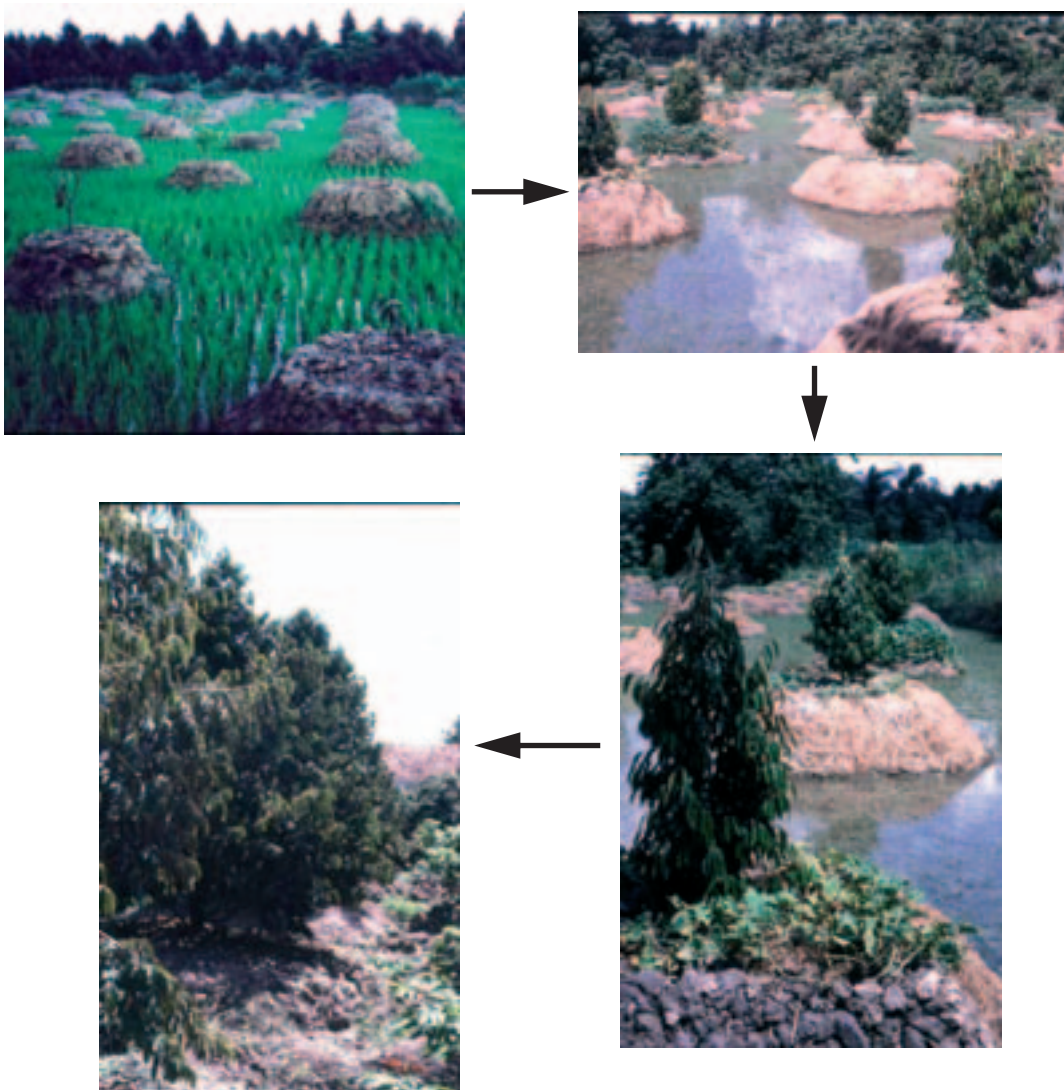


Figure 7.2.1 Transformation of rice paddy to a durian orchard over several years in the Mekong Delta, Vietnam.

Figure 6.6.6), and expose delicate feeder roots to bare soil and damaging solar radiation which leads to poor soil structure in the top soil. This is especially detrimental to many tree species originating from rainforest environments which tend to have rather shallow root systems. If spray irrigation is used, spray nozzles should be directed away from the base of trees to avoid wetting the bark, and around the drip zone where most roots are located. Drip and microjet irrigation uses water efficiently and avoids ponding, but is expensive to install and maintain. Irrigation water should be tested regularly to ensure that it is pathogen-free. Water from rivers and canals that run through orchards are an important source of primary inoculum.

Phytophthora diseases sometimes utilise root and stem damage caused by cyclones and storms. Appropriate windbreaks may help to protect trees from damage as well as disease epidemics. This is particularly important for large, shallow-rooted trees like durians. A severe epidemic of phytophthora patch canker followed a hurricane in south-eastern Thailand in 1994. Evidence is presented in Chapter 4.2 that wounds caused by wind damage attract zoospores and provide entry sites that initiate infections.



Figure 7.2.2 Transplanting of durians into prepared pits at Ba Ria-Vung Tau, Vietnam. These pits are maintained as bare soil and used to contain flood irrigation water during the dry season.

Orchard Management

Soil health

Healthy trees grow from healthy soils. *Phytophthora cinnamomi* causes a devastating dieback disease in the dry sclerophyll forests of south-eastern and south-western Australia, yet is a relatively minor pathogen in nearby wet sclerophyll rainforests or in the tropical highland rainforests of Southeast Asia where it is thought to have evolved (Cook and Baker 1983). A key difference between these ecosystems is the organic-matter content and biological activity of the disease-conducive and disease-suppressive topsoils. *Phytophthora* is a relatively poor saprophytic competitor that struggles to survive in soils rich in organic matter that supports an active and abundant microflora.

The Ashburner system developed in Australia attempts to simulate disease-suppressive soils in horticulture by increasing soil biological activity and biodiversity (Pegg 1977; Baker 1978; Cook and Baker 1983; Erwin and Ribeiro 1996). An annual cycle is established before transplanting, where a green manure crop, such as lupin, is planted at the end of the wet season, then slashed and lightly incorporated with chicken manure and nitrogen-phosphorus-potassium (NPK) fertiliser in spring. Lablab, corn or sorghum is planted over the wet season, then again slashed with chicken manure and NPK fertiliser, followed by lupins in the dry season, *ad infinitum*. Dolomite lime is added to maintain soil pH around 6.0. The cycle is continued for several years until the orchard is established and leaf litter, supplemented with straw and chicken manure, maintains the level of soil organic matter (Figure 7.2.3). This cycle continually replenishes the soil organic matter without disturbing surface roots and provides a mulch layer that dampens soil surface temperatures and preserves soil moisture.

The Ashburner system provides an excellent example of how to manage a healthy orchard in the presence of *Phytophthora*-infested soils, and can be readily adapted to other tree cropping systems. Konam and Guest (2002) showed that cocoa leaf-litter mulches stimulate antagonists and provide a physical barrier for rainsplash inoculum, reducing the incidence of black pod. Chicken-manure amendments are more effective at suppressing *Phytophthora* than other manures (Broadbent and Baker 1975; Aryantha et al. 2000).

Antagonists such as *Trichoderma*, *Gliocladium*, *Bacillus* and *Streptomyces* may be effective in controlled nursery environments, but are generally much less impressive in field trials. The effect of

these biological-control agents is enhanced by other measures aimed to improve soil health and organic matter. Biological control appears to be more effective following improvements to soil health, such as organic-matter amendments that stimulate indigenous suppressive microbes, than by simply adding beneficial microbes to poor soils.



Figure 7.2.3 Young durian trees mulched with straw, Cape Kimberley, Australia.

Fertiliser and water management

The basic aim of water and nutrient management in orchards is to encourage healthy vegetative growth and the sustainable production of high-quality fruit. The precise phenology of tree growth and seasonal variations in water and nutrient requirements must be studied and understood in each environment.

Some durian growers in northern Queensland report that overuse of inorganic fertilisers exacerbates diseases in durian caused by *P. palmivora*, while mulches and manures improve tree health. Tan (2000) studied the effects of a liquid inorganic fertiliser and composted chicken manure on the development of *P. palmivora* diseases in papaya and durian and concluded that indeed chicken manure significantly reduced disease incidence and severity compared to the use of inorganic fertilisers.

The survival of inoculated papaya seedlings was greater in soils amended with composted chicken

manure than in soils that received double the recommended rate of inorganic fertiliser. Root rot occurred in all treatments, however root regeneration occurred in the chicken-manure treatment but not in the inorganic-fertiliser treatments. One hundred per cent of 12-month-old durian seedlings planted in *P. palmivora*-infested, chicken-manure-amended potting mix survived, and the pathogen was eradicated from the soil. In unamended potting mix, the seedlings also survived but the pathogen could be re-isolated from the soil at the end of the experiment. The pathogen was readily isolated after one month from soils that had received regular applications of inorganic fertiliser, by which time all durian seedlings had died (Table 7.2.1).

The survival of the durian in, and the eradication of *P. palmivora* from, chicken-manure-amended potting mix coincided with the stimulation of microorganisms antagonistic to the pathogen that were introduced to the potting mix in the chicken manure. The amendment of potting mix with composted chicken manure led to higher biological activity, and levels of actinomycetes, endospore-forming bacteria and fluorescent pseudomonads over a 3-month period than in potting mix that received regular applications of inorganic fertiliser.

The study reinforces the value of chicken manure as a source of nutrients and biocontrol agents for *Phytophthora* spp. and supports the hypothesis of the growers in northern Queensland that over-fertilisation with inorganic fertilisers may exacerbate disease in durian caused by *Phytophthora*.

Canopy management

Canopy management is also important because it enables farmers to reduce the relative humidity in the canopy, and to remove potential sources of inoculum. Regular harvesting of cocoa, for example, reduces secondary inoculum and is an important component of integrated disease management.

A complete understanding of the disease cycle reveals the importance of orchard hygiene. Diseased plant material, prunings, discarded fruit or unusable parts of fruit are significant sources of inoculum.

Table 7.2.1 Survival of durian seedlings and *Phytophthora palmivora* in potting mix following one month of inorganic or organic fertiliser application.

Treatment		Surviving seedlings (%)	Re-isolation of <i>P. palmivora</i> (%)
Inoculation	Fertiliser		
none	none	100	0
+ <i>P. palmivora</i>	none	100	22.2
+ <i>P. palmivora</i>	2 × inorganic	0	100
+ <i>P. palmivora</i>	2.5% chicken manure	100	0

Orchard hygiene, if implemented rigorously and consistently, can significantly reduce disease pressure in orchards and on farms and, in some cases, may be all that is required to manage diseases caused by *Phytophthora*. More commonly though, hygiene is one essential component of an integrated disease management package (Chapter 8.7).

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7.3 The Use of Mounds and Organic and Plastic Mulches for the Management of Phytophthora Root Rot of Papaya in Northern Queensland

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Abstract

Options for the control of root rot of papaya caused by *Phytophthora palmivora* were evaluated in a field experiment in northerly parts of Queensland, Australia. In the experiment, growing papaya on 0.75 m mounds reduced the incidence of root rot by 38.4% and significantly increased fruit yield. Soil covers of 2 m wide plastic mulch and organic mulch, in combination with 0.75 m mounds, further reduced plant losses by 20 and 10%, respectively. Plastic mulch on flat ground was as effective as the mounded treatments in reducing the incidence of root rot and increasing yield.

Introduction

The northern Queensland papaya industry (latitudes 16°48'–17°26'S), which includes 90% of all papaya (*Carica papaya*) grown in Australia, consists mainly of farms of no more than 2 ha. However, the soil-borne pathogen *Phytophthora palmivora* Butler, which causes a decay of the taproot and eventual death of plants, is widespread in the growing area (Vawdrey 2001). Recommendations for the control of the disease involve papaya being planted on land not previously planted to papaya (Chay-Prove 2000). This situation has been a major constraint to the expansion of the papaya industry in the region.

The conventional method of growing papaya in all growing areas has involved planting seedlings into flat ground (Dunn 2001). Duniway (1979) concluded that the most important environmental factor influencing phytophthora-related root disease was the duration of saturation or near-saturation of soil.

Soil conditions such as these are known to favour the rapid formation of sporangia and infectious zoospores and a high level of disease. Although the most suitable papaya-growing soils in northern Queensland are well-drained loams, these soils are likely to remain saturated for prolonged periods during severe wet seasons. Improving soil drainage through mounding and mulch application has been used successfully in avocado to manage root rot caused by *Phytophthora cinnamomi* (Broadley 1992; Pegg and Whiley 1987).

This study reports on a field experiment that examined the effectiveness of mounds and organic and plastic mulches, with and without the chemical metalaxyl, in reducing root rot of papaya. The experiment was located at a site on a grower's property where *P. palmivora* had been recovered from papaya plants severely affected with root rot.

Methods

Site description and experimental design

The experiment was established on 13 January 1997 in a kraznozom soil on a commercial papaya property at Innisfail, Queensland, Australia. The experiment was set up as a split/split plot in a randomised complete block design. There were

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three replicates each with two whole plots to which the mounding/flat ground treatments were applied. Each whole plot was divided into 3 subplots to which the cover treatments (1) plastic mulch, (2) organic mulch or (3) nil cover were applied. Each subplot was then divided into 2 sub-subplots where (a) metalaxyl or (b) a nil treatment was applied. There were 10 datum plants and 2 guard plants per sub-subplot.

Treatment application

On the 8 January 1997 the experimental site was deep-ripped and rotary-hoed, and mounds (0.75 m high), each 1.5 m wide and 18 m long, were formed in the appropriate plots. Metalaxyl (Ridomil, 50 g/kg) treatments were broadcast evenly on the surface of the beds and lightly raked into the soil just before the application of the soil-surface mulches. Plots treated with organic mulch were covered to a depth of 7.5 cm with composted shredded tree bark obtained from the local council waste depot. The plastic mulch treatments (Table 7.3.1), consisting of 2 m wide black plastic sheets, were laid and then painted white to prevent sunscald damage to the newly planted seedlings.

Plant establishment

Eight-week-old papaya seedlings (Hybrid 29) were transplanted from pasteurised potting mix into flat beds in the experimental area on 13 January 1997. Plants were thinned to 1 per position at flowering when the sex of the plant could be determined. Plants were irrigated as required using dripper lines positioned either side of the planting line. All plots received a basal fertiliser application of Crop King 55[®] (13.2% N, 14.7% P, 12.3% K, 1.5% S), and superphosphate (8.8% P, 20% Ca, 11% S), at rates of 55 and 110 kg/ha, respectively, and dolomite (16.5% CaCO₃ and 10% MgCO₃) at 1100 kg/ha, and two

applications of urea (39 kg/ha) through the irrigation system during the growing of the crop.

Data collection

Plant heights (cm) were recorded at 8, 13 and 17 weeks after transplanting. Plant infection counts were recorded as plants showed symptoms of wilting resulting from the decay of the taproot. Diseased plants were cut at ground level and moved to the inter-row. Samples of diseased roots and stems were obtained from each root-rot-affected plant to identify the causal organism. Sections of diseased roots and stems were surface sterilised in 70% ethanol for 1 minute, blotted dry with sterile paper then transferred to PDA plus 50 mg/L streptomycin sulfate, and the *Phytophthora* selective medium P₁₀ARP+H (Jeffers and Martin 1986). On 6 November, fruit with a diameter greater than 7.0 cm was harvested and the total fruit number and total fruit weight per plot assessed.

Results

Some seedlings died within 1–2 weeks of transplanting. *Rhizoctonia solani* was recovered from basal stem lesions on a few plants using PDA plus streptomycin sulfate culture medium, but the cause of most plant deaths was most likely due to physical damage to the taproot at transplanting. Planting sites where all plants had died were replanted within 4 weeks of the initial transplanting.

By 11 March, there were quantitative differences in plant growth between treatments (Table 7.3.1). Assessments conducted on 11 March and 22 April showed a significant mound × soil cover interaction, with the height of plants grown on flat ground with organic mulch significantly reduced ($P < 0.05$) compared with all other treatments. The pre-plant application of metalaxyl had no effect on plant growth ($P > 0.05$) except in the assessment conducted

Table 7.3.1 Plant heights of papaya grown on mounds or flat ground, with organic and plastic mulches.

Treatment	Plant height (cm) ^a		
	11 March	22 April	20 May
Mound/plastic mulch	78.0 a	119.0 ab	176.0 a
Mound/organic mulch	67.0 a	95.0 c	142.0 abcd
Mound/bare soil	88.0 a	107.0 abc	137.0 bcd
Flat/plastic mulch	68.0 a	109.0 abc	161.0 ab
Flat/organic mulch	46.0 b	65.0 d	101.0 e
Flat/bare soil	73.0 a	88.0 c	108.0 de

^a Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

on 22 April, where the chemical improved plant growth ($P < 0.05$) when applied to mounded soil with organic mulch. In this assessment, plant heights were 123 cm in mounded plots treated with organic mulch and metalaxyl, compared with 95 cm in mounded plots with organic mulch alone. The final assessment, conducted on 20 May, showed a significant mound \times soil cover interaction, with a significant increase in plant height ($P < 0.05$) in mounded plots with both organic and plastic mulch compared with mounded plots with bare soil. Plants grown on mounds with and without mulches, and on flat ground with plastic mulch, were taller ($P < 0.05$) than plants grown on flat ground with organic mulch or bare soil.

At the conclusion of the experiment, the use of mounds was shown to be very effective at reducing the incidence of root rot (Figure 7.3.1). The percentage of plants with root rot was significantly greater ($P <$

0.05) in plots where plants were grown on flat ground with either organic mulch or bare soil compared with plants grown on mounds. There was no difference in survival ($P > 0.05$) between plants grown on flat ground with plastic mulch and plants grown on mounds. The pre-plant application of metalaxyl granules had no effect ($P > 0.05$) on reducing the incidence of root rot. *Phytophthora palmivora* was recovered from all root rot affected plants.

Larger, more mature fruit was obtained from larger, more vigorous plants, and fruit weight varied across the various treatments (Figure 7.3.2). Significantly heavier ($P < 0.05$) fruits were harvested from plants grown on mounds, and on flat ground with plastic mulch, than from plants grown on flat ground with organic mulch or bare soil. The highest yield was obtained from plants grown on mounds with plastic mulch.

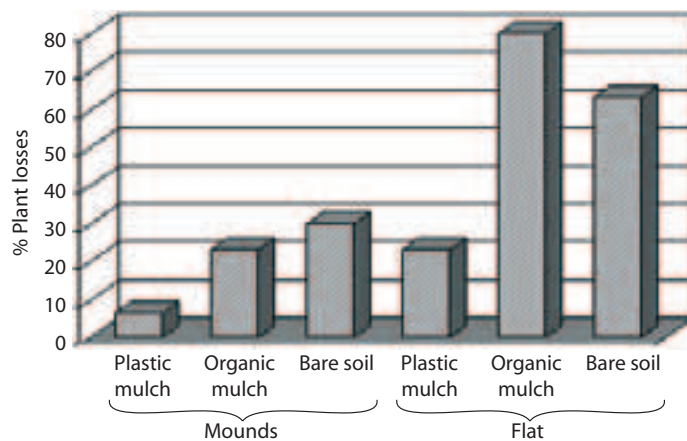


Figure 7.3.1 Effect of growing papaya on mounds or flat ground with or without organic or plastic mulches, on the incidence of phytophthora root rot and plant losses.

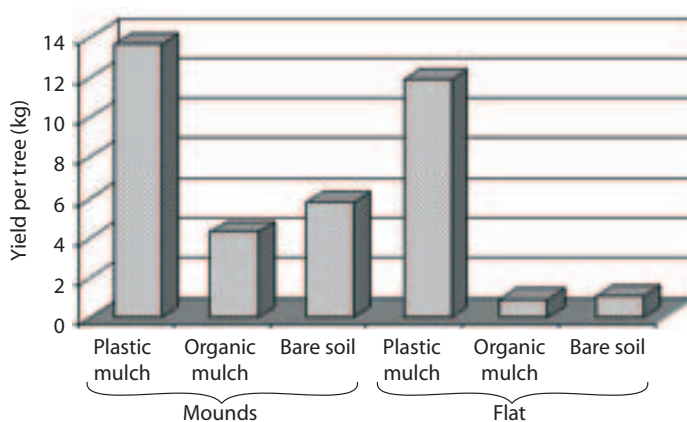


Figure 7.3.2 Effect on yield of growing papaya on mounds or flat ground with or without organic or plastic mulches.

Discussion

In field situations where a soil-borne disease is well established, growers are generally encouraged to create a growing environment that is favourable for the host and less favourable for the pathogen. The persistence of free water in the soil has a major influence on the development of phytophthora-related disease as it favours the increase in *Phytophthora* populations (Duniway 1979). Therefore, optimising vertical drainage should effectively reduce the period of soil saturation and subsequent damage due to disease (Duniway 1983). The use of mounds in our field experiment achieved this result by reducing plant losses due to root rot and substantially increasing fruit yield.

Wide plastic mulch also reduced plant losses and increased fruit yield in both mounded and non-mounded plantings. This result was most likely due to reduced water infiltration into the soil rather than solarisation, as the plastic was painted white before transplanting, and the predominantly overcast conditions at that time of year would have reduced the heating effect. However, the cost of purchasing and laying plastic mulch, and environmental concerns about its disposal, are likely to prohibit its use. The use of shredded tree bark as organic mulch caused severe plant losses due to root rot, and substantially reduced fruit yield in all but mounded plots. This result was most likely due to increased soil moisture retention and the positive influence this has on increasing disease development (Vawdrey et al. 2002). Other types of organic mulch may be more effective, for example some types of bark suppress *Phytophthora*, while leaf litter, straws and manures may improve drainage as well as suppress the pathogen (Konam and Guest 2002; Ribeiro and Linderman 1991). Future research will evaluate the integration of single row mounds and foliar applications of potassium phosphonate for the management of phytophthora root rot of papaya.

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7.4 Root Infusion of Phosphorous Acid for the Control of Phytophthora Foot Rot in Black Pepper (*Piper nigrum* L.)

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Abstract

Phytophthora foot rot caused by *Phytophthora capsici* is the most devastating disease of pepper (*Piper nigrum* L.) in Sarawak. This paper outlines the symptoms and management of the disease. The application of phosphorous acid by the root infusion technique is described and its advantages over conventional application methods are discussed.

Introduction

Pepper (*Piper nigrum* L.), which is popularly known as the 'king of spices', is the most important spice crop grown in Sarawak. Sarawak is the main producing state in Malaysia, contributing about 98% of the country's total production. It exported about 26,000 tonnes in 2001 valued at USD45m according to Sarawak's Department of Statistics. In Sarawak, pepper is cultivated as a monocrop in smallholdings with an area of 13,000 ha. Though the crop is planted throughout the state, the main areas are largely concentrated in the central and southwestern parts.

Pepper cultivation in Sarawak is affected by a number of fungal diseases that cause heavy losses in yield and reduce the economic lifespan of pepper vines. Among these diseases, foot rot caused by *P. capsici* is the most important and devastating.

Symptoms

Phytophthora can infect both mature and immature plants, and symptoms of the disease may appear on all parts of the plant. The infection of pepper starts at the collar region of the vine. However, it is usually

not detected until the top portion of the vine shows signs of leaf yellowing and wilting, and the branches appear to droop. Once these symptoms are noticed, the infection is already advanced, with the underground stem having brownish-black lesions and extensive rotting of the roots. The lesion may extend upwards along the main stem of the vine. Infected berries turn brown, have a sunken appearance and may drop. As the disease progresses, leaves and branches turn brown. The shedding of leaves and breaking off of branches follows until only a skeleton of the vine remains.

Though the pathogen is soil borne and infection usually starts at the collar region or the underground part of the vine, aerial infection due to wind dispersal and rain-splash of spores sometimes occurs. In this instance, characteristic fimbriate-edged leaf lesions are observed on the leaves.

Disease Management

At present there are no pepper cultivars with high levels of resistance to *P. capsici*. An integrated approach consisting of both cultural and chemical methods is needed to manage this disease.

As foot rot spreads very rapidly and the symptoms take some time to develop, it is difficult to control the disease. Therefore, the management of this disease needs to emphasise prevention based on good cultural practices.

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Field hygiene such as cleaning of farm tools and equipment should be practised. Eliminating the movement of infected soil into disease-free areas will prevent dissemination of the pathogen. Pruning of the lower branches that are in contact with the soil is recommended, especially during the rainy season. Field sanitation by roguing infected plants to prevent inoculum build-up and spread of the disease is also recommended. The garden should have proper drainage to prevent excess soil water and waterlogging, which is conducive for the development and spread of the zoospores. The use of planting materials from diseased gardens or high-risk areas should be avoided. It is important to be vigilant, so that prompt action can be taken to contain outbreaks.

Chemical control is an important component in managing the disease, especially when disease symptoms start to appear. Fungicides such as copper, fosetyl-aluminium or metalaxyl are being used. Kueh (1993) and Kueh et al. (1993) recommended control of the disease by the use of metalaxyl or phosphorous acid, applied either by foliar spraying, soil drenching or trunk injection. However, the conventional method of spraying is unsatisfactory due to wet weather conditions at the end of the year when the disease incidence is highest. The effect of soil drenches is short-lived

because phosphorous acid is oxidised by soil microorganisms that render it non-fungicidal (Whiley et al. 1987). Trunk injection causes injury to the vine and predisposes the plant to other pests and diseases. An alternative mode of applying phosphorous acid to control phytophthora foot rot was therefore developed.

Root Infusion Technique

This aim of the root infusion technique is to increase the level of phosphonate in the root and vine tissue, which renders these plant parts increasingly tolerant to invasion by *Phytophthora*.

For successful implementation of the root infusion technique, the choice of root is important. The primary root chosen must be without any damage or wounds (Figure 7.4.1), and should be about 7.5–10 mm in diameter. The soil on the mound is dug out carefully with a hand spade. Following the direction of a primary root, the surrounding soil is loosened to isolate the root. After a suitable root is isolated, other secondary roots or rootlets on the primary root are trimmed off and soil on the root surface is also removed. The root is then cut with a sharp knife. The cut end is immediately inserted into an 80–100 mL plastic bottle that has been filled with 1–2% phosphorous acid (Figure 7.4.2). The root must reach



Figure 7.4.1 A primary root isolated for infusion



Figure 7.4.2 Root infusion in progress

the bottom of the bottle so that the acid can be absorbed. To keep the bottle in place at an angle, the surrounding soil is pushed and pressed near the bottle. Each vine should at least take up half the volume of the diluted acid.

The treatment is usually carried out in the morning, up until midday, as translocation is generally stronger that time of the day. While the treatment is in progress, each vine is checked to ensure that there is absorption. If there is no uptake, or the volume taken up is too low, the root should be replaced with another one. The time taken for complete absorption varies from vine to vine, with a range of 1 to 4 hours. After the treatment, the bottle is removed and the root is re-covered with soil.

Preliminary studies on the application of phosphorous acid through root infusion showed that disease spread was impeded and the productive life span of vines in the infected garden extended (Wong and Wong 1996).

Advantages of the Technique

The application of phosphorous acid by the root infusion technique has many advantages over conventional methods of application.

- No wastage. The phosphorous acid that is absorbed by the root is directly translocated in the plant and, as a result, there is no chemical drift or spillage to non-target area causing excessive wastage of chemical. With no unnecessary loss of chemical, the quantity required is less and this brings cost savings.
- Less chemical hazard. As there is no problem of chemical drift, the risk of the operator being exposed to the chemical is reduced. In addition, phosphorous acid is a non-toxic compound, which further enhances the operator's safety.
- Protected from rain. As the phosphorous acid is infused through the root, it is protected from being washed off if rain follows the application.
- No environmental pollution and no interference with soil microorganisms. Foliar spraying and soil drenching of chemicals cause air pollution and contaminate the soil. These modes of application can also cause injury to non-target plants and are detrimental to soil microorganisms. Root infusion involves the direct absorption of phosphorous acid and therefore these problems do not arise.
- No damage to the vine. Though trunk injection is a popular way of administering phosphorous acid in many crops, it was found to be unsuitable for pepper vines. Drilling the stem causes injury that might predispose the plant to other pathogens.

Injection technology and injectors have been developed for trunk injection where longer diameter injection holes are not a problem. There is no physical damage observed in the vine when root infusion has been used.

- Simple tools and technique. This technique does not require any expensive or sophisticated tools, only plastic bottles. In cases when plastic bottles are not available, plastic bags or used cans could be used to improvise. The application technique is simple and easy to implement.

Minor disadvantages include the labour intensiveness and the problem of finding suitable roots, especially in gravel soils and when roots are already diseased. This techniques has also been used to threat phytophthora diseases in other plants, including coconut, but is especially suitable to perennial vines.

Conclusion

Integrated disease management strategies with emphasis on preventive control should be adopted to manage phytophthora foot rot. Apart from good cultural practices, which are of the utmost importance in preventing the disease, chemicals such as phosphorous acid protect plants against infection. The root infusion technique has been shown to be a more efficient way of delivering phosphorous acid in the case of pepper vine. With its various advantages, this improved mode of application offers a practical alternative over other application methods and is an attractive economic proposition for the small pepper farmers.

Acknowledgment

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7.5 Biological Control of Black Pod Disease on Cocoa in Malaysia

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Abstract

In order to reduce losses due to black pod disease in cocoa, the efficacy of a number of biological control agents has been tested. One approach to biological control is to increase the number of beneficial bacteria on the surface of the cocoa pods. It is recognised that biological control of *Phytophthora palmivora* is just one part of an integrated disease management strategy.

Introduction

Black pod disease caused by *Phytophthora palmivora* is one of the most important diseases of cocoa (*Theobroma cacao* L.) in Malaysia. The major economic losses are from the infection of the pod. Losses caused by black pod disease in Malaysia are estimated to be less than 5%, but at certain times could be over 70% (Tey and Bong 1990; Bong and Stephen 1999). Normally, black pod disease can infect cocoa pods at any stage of pod development, but the most significant economic losses arise from infection of the immature pods. Temperatures of between 15 and 30°C, relative humidities of 80 to 100% and high rainfall constitute conditions conducive for disease development. The management of this disease in Malaysia relies heavily on chemical control, which can be costly and labour intensive. Changing community attitudes towards the use of pesticides are driving a need for alternative approaches to the control of black pod disease. This paper discusses the current practices and the progress made in some of the research conducted at the Malaysian Cocoa Board towards sustainable management of black pod disease of cocoa.

Biological Control

Biological control may offer an environmental friendly approach to the management of plant diseases and can be combined with cultural and physical controls and limited chemical usage for effective integrated disease management systems. Biological control avoids problems experienced with chemical controls, such as the development of chemical resistance in the pathogen. Biological control cannot completely eliminate the pathogen, may not work as rapidly as chemical methods and may provide only a partial level of control. Biological control also can be an important component in the development of sustainable agriculture management systems. Biological control includes the use of resistant varieties and the manipulation of biological competitors and antagonists.

Biological control agents isolated from healthy cocoa pods and the infected pod surface (resident antagonist) can interfere with the growth of the pathogen. Epiphytic microorganisms, especially bacteria, are capable of inhibiting the growth of *P. palmivora* (Bong et al. 1998; Bong and Stephen 1999). The humid conditions in which cocoa is cultivated provide a favourable environment for the development and survival of epiphytic microorganisms antagonistic to *P. palmivora* (Galindo 1992). Bacteria have been favoured because they are easy to handle, have a high reproductive rate and are the first colonisers of the phylloplane (Spurr and Knudsen 1985).

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Screening for Resistance to *Phytophthora*

Recently, the focus of cocoa breeding by the Malaysian Cocoa Board has placed a high importance on black pod resistance. The Malaysian Cocoa Board is currently involved in international collaborative research programs with the Common Fund for Commodities (CFC)/International Cocoa Organisation (ICCO)/International Plant Genetic Resource Institute (IPGRI). The aim of these programs is to screen cocoa germplasm for resistance to *P. palmivora* through leaf disc and detached pod tests. Screening for resistance to black pod was devised and adapted from methods published elsewhere (Nyasse et al. 1995) in order to come up with a cheap and rapid leaf inoculation method for preliminary mass screening. It is also being used in host-pathogen interaction studies to compare the aggressiveness of various isolates of the pathogen and for determining the presence of a specific host-pathogen interaction, which is important in the deployment and management of black pod resistance in the host. A significant host-pathogen interaction was found, with some cocoa clones being more susceptible to some *P. palmivora* isolates and less sensitive to others.

Numerous imported cocoa clones of the PBC, QH, SDS, UP and KKM series, and other local selections developed by various agencies and plantations, were tested against two *P. palmivora* isolates. Those found to have resistance comparable to or greater than that of PBC123, based on the leaf inoculation test, included BR25, K82 and P7. Others were consistently found to be more resistant than PBC123 to black pod phytophthora in leaf inoculation tests.

Resistance of rootstock to *Phytophthora* is also an important consideration in clonal plantings. A simple method that can be used to screen for resistant rootstock entails coating the seeds with the *P. palmivora* sporangia before germinating them (Ahmad Kamil and Yahya 2000). This is a destructive method for the selection of resistant rootstock and elimination of susceptible ones, a consideration not insignificant to the breeders. A start has been made in establishing a pool of resistant rootstock for breeders to further develop and form the basis of a study on compatibility of stock-scion interactions. Most of the rootstocks in recent new plantings has been derived from the most readily available source, seeds of PBC123 or BR25 obtained from commercial cocoa plantations. As observed in germination tests of seeds from open-pollinated pods of over 20 clones and hybrids (KKM 22, BAL 244, QH series of clones, TT 1, Desa

series, BR 25, PBC 123, UIT1 × EQX107, SDS18, PA300 × K82, EET 390 × K 82, PA20 × IMC 23, UIT1 × NA33 and LS4), germination rates of seeds coated with *P. palmivora* could be as high as 90% or as low as under 30% depending on the concentration of inoculum and the resistance to infection of the seeds (Ahmad Kamil and Yahya 2001).

Development of Microbial Biocontrol Agents

The application of chemical control in the management of cocoa diseases is mainly practised in the control of black pod, which often shows explosive epidemics. In view of rising consumer concern with the environment and health, and the fact that premium prices are paid for organically grown products, the potential for environmental friendly and sustainable biological control methods using beneficial microbes to combat pathogens has been investigated. Fungal and bacterial antagonists were collected from the rhizosphere and phylloplane of cocoa. Recent research conducted in Sabah revealed that certain bacteria and fungi isolated from the surfaces of healthy and infected cocoa pods are antagonistic to *P. palmivora*. They include: *Gliocladium virens*, *Trichoderma harzianum*, *Pseudomonas putida* biotype A, *P. aeruginosa*, *P. spinosa*, *Burkholderia gladioli*, *Burkholderia* sp., *Bacillus sphaericus*, *B. polymyxa*, and *Serratia marcescens* (Bong et al. 1998; Ahmad Kamil and Yahya 1999; Bong and Stephen 1999; Shari Fuddin 1999). The fungal and bacterial antagonists selected for further study are screened for pathogenicity towards plants and animals. Two potential fungal and bacterial species are being further evaluated, and are now into their second season of field evaluation for efficacy in control of black pod trials established in Lahad Datu, Sabah. Introduced during the cropping period in the first season of the trial, the black pod incidence in treated plots was significantly lower than in the control. In terms of the effect on the progress of the epidemic of black pod, based on comparison of the apparent rates of infection, plots treated with the antagonists showed infection rates half those in the control (Figure 7.5.1). Hence, there is potential for further investigations. Research findings also demonstrated that the biocontrol agents could be produced in liquid culture. The use of bio-fermentation for mass production of biocontrol agents needs to be cost-effective, and they should cost less than chemicals. The method of application depends on the mode of action of bacteria and should be compatible with established crop-management practices.

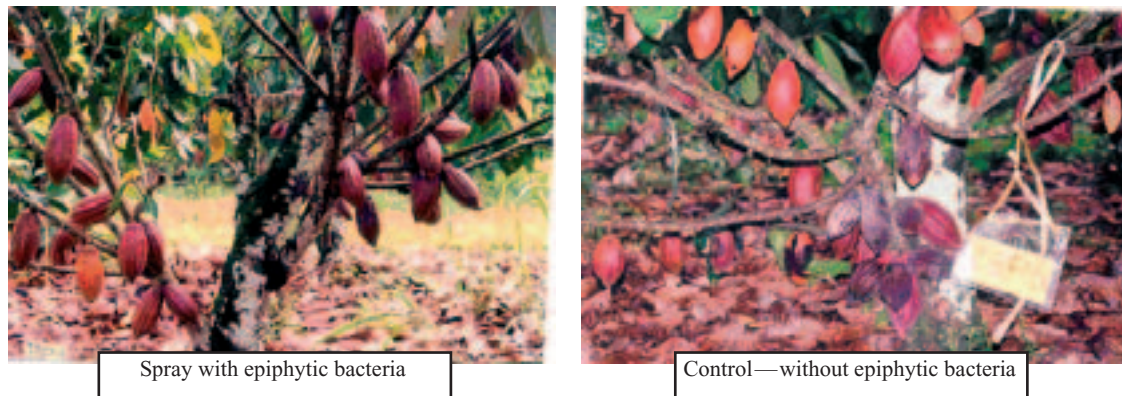


Figure 7.5.1 Field experimentation on the use of antagonist epiphytic bacteria in controlling black pod disease caused by *Phytophthora palmivora*.

Diversity of Microorganisms and Their Roles in the Cocoa-based Agro-ecosystem

Among the microflora found on cocoa are both pathogens and beneficial microorganisms, the potential of most of which to act as a biocontrol agent has yet to be determined (Bong et al. 1998). It is important to know what is present, in order to improve the effectiveness of integrated disease management. Present in the soil, and in the cocoa rhizosphere in particular, are beneficial fungi and bacteria that may be effective antagonists of *Phytophthora*. As mentioned previously, many beneficial bacteria, particularly species of *Pseudomonas*, are resident microbes on pod surfaces.

Basic research is also conducted on microorganisms that have potential use in ecosystem-based disease management strategies for cocoa. Basic studies in this area are focused on the environmental influence on the growth of the pathogens and/or beneficial microbes. The optimal range of temperature for growth of the bacterial antagonists investigated was found to be 28–35°C, though a few species are thermophilic, surviving at temperatures up to 55°C. Most of the bacterial antagonists grow well at above pH4. Clearly, the key is to improve persistence and survival of biological control agents in the field.

The Outlook for Black Pod Disease Management

From the results of many years of research aimed at controlling black pod disease, one has to conclude that there is no single solution. Better disease control has to be based on a combination of agronomic practices that hinder the development and spread of the pathogen, the use of effective biocontrol agents and more precise timing of spray applications, and

the use of resistant clones. It is also important to understand the range of environmental conditions, such as temperature and moisture, in which biocontrol agents are effective under field conditions. The environment in which cocoa is cultivated provides conditions favourable for epiphytic bacteria as biocontrol agents to multiply rapidly in the field. Host resistance will remain the cornerstone of a more sustainable, user and eco-friendly and less costly integrated disease-management strategy of cocoa with cultural, chemical and microbial control as supporting components.

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8

Phytophthora in Durian



8.1 Botany and Production of Durian (*Durio zibethinus*) in Southeast Asia

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Abstract

Durian originated in wet tropical Southeast Asia, where 30 species have been described. Wild *Durio* spp. are still found in Borneo and Sumatra, although rainforest destruction seriously threatens genetic diversity in the genus. One species, *Durio zibethinus* L., is widely cultivated, primarily for consumption of the fresh fruit, although other species and uses are described. Trees are usually grown in mixed home gardens for domestic consumption. Large-scale commercial orchard cultivation is practised in Thailand, Malaysia and Indonesia, while industries are developing in Vietnam, the Philippines and Australia. The seasonality of production causes significant fluctuations in supply and market prices, and creates opportunities for new plantings and cultural techniques that exploit the low supply of fruit during the off-season.

Origin and Diversity of Durian

The genus *Durio* (Order Malvales, Family Bombacaceae) has a complex taxonomy that has seen the subtraction and addition of many species since it was created by the German botanist Georgius Everhardus Rumphius (1627–1702) in the 17th century. Currently 30 species are recognised, including 9 to 11 species with edible fruit (Lim 1990; Brown 1997; Lim and Luders 1997). However, there are many species for which the fruit has never been collected or fully described and it is likely that other species with edible fruit exist (Brown 1997). The most extensively grown and economically significant species is *Durio zibethinus* L. (Lim 1990; Nanthachai 1994; Brown 1997). Many cultivars and local selections are grown.

The Latin epithet *zibethinus* was given by Linnaeus, sight-unseen, from a description of durian in Rumphius’s posthumously published, classical work on Indonesian flora, *Herbarium Amboinense* (1741–1750), containing an explanation that the fruit was used to bait the civet cat (Brown 1997). Thus, the common misconception that *D. zibethinus* (durian) was named because it smells like the Indian civet cat (Watson 1984) – a feature that no doubt accounts for its Dutch name of ‘Stinkvrucht’ – is false. Brown (1997) also points out that Linnaeus is the correct authority for *Durio zibethinus*, not Murray. He notes that the confusion arose in the 1800’s when a simple error found its way into several major taxonomic works.

Borneo is thought to be the centre of diversity of the genus *Durio* and many species are indigenous to the Malay Archipelago, but over many hundreds of years it has been introduced into Thailand, Vietnam, Laos, Kampuchea, Myanmar (Burma), Sri Lanka, New Guinea, West Indies, Polynesian Islands, Hawaii, Florida, southern China (Hainan Island), and northern Australia (Lim 1990; Nanthachai 1994; Brown 1997; Lim and Luders 1997).

Botany

Durian is a tall evergreen tropical tree with a buttressed base and straight trunk and almost horizontal upper

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branches. In its natural rainforest environment, it can grow to 60 m in height, but rarely exceeds 20 m when grown as grafted clones or rootstock in horticultural settings (Nanthachai 1994). Architecturally, the tree exemplifies Roux's Model with a tall, broadly conical frame tapering to an apex (Figure 8.1.1). The leaves are alternate and lanceolate, 10–15 × 3–5 cm, with a glossy upper surface and velvety, silver–golden lower surface (Figure 8.1.2), due to the dense covering of overlapping peltate and stellate hairs (Brown 1994; see also Chapter 3.2).

There are many excellent descriptions of the physical (Singh and Rao 1963; Davis and Bhattacharya 1974; Watson 1984; Lim 1990; Masri 1991; Nanthachai 1994; Yaacob and Subhadrabandhu 1995; Brown 1997) and micro-morphological characteristics of durian in the literature (Baas 1972; Rao and Singh 1964; Rao and Ramayya 1981; Hasan and Dodd 1989; Salma 1999). Cauliferous inflorescences are borne in clusters of 3–10 flowers over a period of about 2–3 weeks during the dry season (Figure 8.1.3). Pedicels, 5–7 cm long, support globose flower buds 2 cm in diameter, opening to reveal 5–6 cm long, greenish-white flowers. The tubular calyx has three to five triangular teeth surrounding five petals. Stamens are arranged in five bundles around a pubescent style and protruding capitellate stigma. Flowers open late in the afternoon and pollen release is complete before midnight. The stigma remains

receptive until early morning, facilitating pollination by bats and moths. Fruit development is sigmoidal and takes 95–130 days, depending on the species and cultivar. Under normal conditions, fruit ripening heralds the start of the rainy season (Table 8.1.1).

Durian is the most famous fruit in Southeast Asia and is renowned for its strong odour and unique taste. The durian fruit is large (between 2 and 5 kg), pendulous, round to oblong in shape, covered with strong sharp spines, and the pericarp is yellow–green to green or brown in colour and does not change significantly with ripening (Figure 8.1.4). Commercial orchards focus on a few popular cultivars and aim to produce medium-size fruit of about 2.5 kg in weight. The fruit usually comprises five locules, holding one to seven large brown seeds covered in the edible flesh (aril), which is cream to yellow in colour, depending on the variety (Figure 8.1.5; Lim 1990; Tinggal et al. 1994). The arils typically comprise 20–35% of the fruit weight, and are composed of 2.5% protein, 2.5% fat, 28% carbohydrate and 67% water, with smaller amounts of fibre, minerals and vitamins. The odour originates from a complex mixture of thiols, esters, ethers and sulfides.

Durian fruit is preserved by freezing, or in the form of a paste or cake that is used to flavour ice-cream, bread or pastries, or the fruit can be fermented,



Figure 8.1.1 Shape of the mature durian tree, showing the tall, straight, buttressed trunk tapering to a conical apex.



Figure 8.1.2 Lanceolate shape and distinct upper (a) and lower (b) surfaces of durian leaves.

salted or boiled in sugar syrup. Pre-packaged frozen durian arils, usually from Thailand, are becoming widely available in Asian supermarkets in Western countries, including Australia.

The seeds are sometimes eaten after boiling or roasting and young shoots and immature fruit can be cooked as vegetables. Fresh seed germinates within 3–8 days to produce a fast-growing seedling that shows strong apical dominance.

Fruit rind can be dried and used as a fuel. The wood is coarse, lightweight and is used in light construction and to make furniture and clogs, although it is not durable and is rarely used for construction (Lim 1990; Brown, 1994; Nanthachai 1994; Brown 1997).

Fruit Production

Durian is strictly tropical and stops growing when mean daily temperatures drop below 22°C, which occurs frequently at the extremes of cultivation in Thailand and Queensland (Nanthachai 1994).

Annual rainfall of 1500 mm or more is required and supplementary irrigation may be necessary during the dry season. The tree prefers deep, well-drained loamy soils but is vulnerable to uprooting and damage during storms and cyclones and requires protection from strong winds.

Flowers are borne mostly on horizontal limbs and pruning is used to limit the number of plagiotropic limbs and to limit tree height. Flowering is naturally stimulated by the onset of the dry season, or can be induced out-of-season after drying the soil by covering with plastic sheets (Figure 8.1.6), or through the use of growth regulators. Flower buds are thinned, and fruitlets are thinned again to optimise the size of mature fruit and to remove fruit that are too high or at the extremities of lateral branches, as the weight of mature fruit is likely to cause branches to break.

Harvesting involves many challenges due to the height of the tree and weight and spikiness of the fruit. Ripe fruit falls to the ground, but is usually



Figure 8.1.3 Clusters of durian inflorescences.



Figure 8.1.5 Aril colour of *Durio zibethinus*.



Figure 8.1.4 Mature durian fruit.



Figure 8.1.6 Plastic mulches are used to induce out-of-season flowering (Ben Tre Province, Vietnam).

damaged in the process. Farmers sometimes tie near-ripe fruit to the branch so that it detaches but does not fall and can be harvested without damage (Figure 8.1.7a). Another method involves one harvester climbing the tree and dislodging ripe fruit while others hold a net underneath, catching dislodged fruit before it hits the ground (Figure 8.1.7b). Yields are erratic and variable, however the best orchards in Thailand produce 50 fruit per tree, or 10–18 t/ha, each year.

Durian Cultivation in Southeast Asia and Australia

Durian has been cultivated for centuries at the village level — probably since the late 1700s, and commercially in Thailand since the mid 1900s (Alim et al. 1994). Since the early 1990s, the domestic and international demand for durian in the Association of South-East Asian Nations (ASEAN) region has increased dramatically, due in part to the rising wave of affluence in Asia (Nanthachai 1994; Lim and Luders 1997). Limited supply has driven a rapid expansion of the area under cultivation, particularly in Thailand, Malaysia and the Philippines (Alim et al. 1994; Nanthachai 1994). By 1997, the value of the industry worldwide was conservatively estimated at USD1.5 billion (Lim 1998). Durian is an economically and culturally important crop in Thailand, Malaysia, Indonesia, Brunei, Vietnam, Myanmar, Cambodia and Lao People's Democratic Republic (Alim et al. 1994; Lim and Luders 1997; Table 8.1.1). The leading exporters of durian in the world are Thailand, Malaysia and Indonesia in descending order, while the Philippines and Vietnam also produce durian for

domestic consumption (Alim et al. 1994; Lim and Luders 1997; Dr Nguyen Minh Chau, pers. comm.). Malaysia still imports a significant amount of durian in its off-season. Durian was introduced to Australia in 1975 by a small number of tropical fruit enthusiasts, and orchard plantings commenced in 1980 in northern Queensland and in 1984 in Darwin (Zappala and Zappala 1994), although it remains a boutique industry.

The majority of production occurs in short seasons of two or three months, although there are two fruiting seasons in Malaysia and Indonesia because the fruit is grown in different localities affected by either the north-east or north-west monsoon. Production in Thailand, Peninsular Malaysia, Kalimantan and Sulawesi is highest between June and July, while harvest peaks in the Philippines in August–November, and Sabah, Sarawak, Java and northern Australia between October to February (Table 8.1.2; see also Alim et al. 1994; Graef and Klotzbach 1995; Brown 1997). The seasonality of durian generates significant opportunities for trade between areas where the fruit is in season and areas where it is not, or in cities and non-producing countries.

Indonesia

Most of the fruit is produced in Java, Sumatra, Kalimantan and Sulawesi (Alim et al. 1994). Indonesia exported 331 t of durian in 1993 — its main market being Singapore (Graef and Klotzbach 1995). Indonesia's durian industry is concentrated on Sumatra, Java and to a lesser degree Kalimantan. In 1992, the area planted was estimated to be 36,000 ha with production of 152,500 t (Alim et al. 1994).



Figure 8.1.7 Methods of preventing damage to mature fruit: (left) tied fruit; (right) catching fruit.

Table 8.1.1 Seasonality of durian harvests (shaded) in durian-producing regions (Lim and Luders 1997).

Production area	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Western Malaysia												
Eastern Malaysia												
Thailand												
Indonesia												
Vietnam												
Lao PDR												
Cambodia												
The Philippines												
Brunei												
Myanmar												
Singapore												
Northern Queensland												
Northern Territory												

Table 8.1.2 Production of durian in selected Southeast Asian countries.

Country, year and reference	Area planted (ha)	Production (t)	Value (USD million)	Major markets
Indonesia, 1992 (Alim et al. 1994)	36,024	152,000	36-780	Singapore
Malaysia, 1992 (Alim et al. 1994)	61,000	384,000	840-1020	Singapore
Philippines, 1993/94 (Nanthachai 1994)	8000	145,000	325-522	Domestic
Thailand, 1999 (Salakpetch 2000)	138,024	927,200	448-2686	Hong Kong
Vietnam, 1993/94 (Chau 1994 ^a)	10,000	110,000	33-330	Domestic, Taiwan

^a Dr N.M. Chau, pers. comm., 1994

Malaysia

Durian is grown in Peninsular Malaysia, Sarawak and Sabah. Like Thailand, there are more than 200 varieties of durian registered, but only 20 are widely used. Durian has traditionally been produced in small orchards, 0.5-1.0 ha in size, but more recently, 12-120 ha commercial orchards have been established (Alim et al. 1994). In 1991, Malaysia exported USD16.3 million worth of fresh durian, with about 90% going to Singapore (Graef and Klotzbach 1995). Durian fruit is produced in most states of Malaysia and, in 1992, 384,000 t of fruit was produced from the 61,000 ha under cultivation, which comprises 31% of the total area planted to fruit in the nation (Alim et al. 1994). Most of the fruit produced in Sabah and Sarawak is consumed locally, however some fruit is exported to Brunei and Singapore. During the off-season in Malaysia, fruit is imported from Thailand (Lim and Luders 1997).

The Philippines

Durian is a high-value crop with great prospects for export from the Philippines, as the harvest season is later than in other Southeast Asian countries. The

Philippines is actively expanding durian production, especially in the typhoon-free areas in Mindanao. An estimated additional 30,000 ha of durian would be required to meet domestic demand if consumption rose from the current 0.2 kg to 2 kg per capita, let alone the 14 kg per capita consumption in Thailand.

Thailand

The Thai durian industry started in the provinces around Bangkok, but was almost destroyed by a series of catastrophic events in the 1940s and 1950s (Alim et al. 1994). Durian production in Thailand is now concentrated in the east (49% of the total cultivated area, with Chanthaburi and Rayong provinces being the major producers) and south (44% of the total cultivated area, with Chumporn the major producer) of the country. In 1999, Thailand produced 927,200 t of fruit from 138,000 ha of orchards, almost half of the world's durian production. About 5.5% of this is exported as fresh and frozen fruit. In 1993, approximately 10% of exports were frozen product. The main market for fresh durian is Hong Kong, as well as Malaysia,

Taiwan, Canada, the United States of America, Singapore, Brunei, Australia, Japan and Indonesia, representing 80% of the world export trade, worth USD48 million in 1996 (Graef and Klotzbach 1995; Lim and Luders 1997).

In 1995, the area planted to durian was approximately 128,000 ha, which accounts for 11% of the total area planted for fruit production in Thailand. Most of the durian production is based on four commercial cultivars, although there are more than 200 cultivars in use. Flowers are hand-pollinated to improve fruit set and yield. Because of the diversity of cultivars and growing regions, the harvest season spans from April to September, with a constant supply between May and August.

Vietnam

The durian industry in Vietnam is quite small, catering mainly for the domestic market, with some export trade with Taiwan (Dr N.M. Chau, pers. comm.). Durian was introduced to southern Vietnam approximately 30 years ago from Thailand and the Philippines, and is now a key element in the reconstruction of horticulture in the Mekong Delta. In the five-year agricultural strategy of the Vietnamese government (1996–2000), durian was identified as a priority crop. In 1993–1994, Vietnam produced 110,000 t of durian for local consumption from about 10,000 ha, mainly in the lowlands of the Mekong Delta (Tien Giang, Can Tho, Soc Trang, Vinh Long, Ta Vinh and Ben Tre provinces). However, the fruit is also produced on the well-drained soils of the highlands in the south-east (Ho Chi Minh City, Dong Nai, Binh Duong, Lam Dong, Ba Ria Vung Tau provinces), Dak Lak Province in the central highlands, and Thua Thien-Hue Province on the central coast.

In the past, durian orchards were established from seedlings rather than from selected varieties, but grafting onto rootstocks has become more popular. Many of the nurseries that provide the grafted material, however, do not choose the most favourable rootstocks and do not use sterile potting media. In the Mekong Delta, durian seedlings are established in raised beds, while in the south-east, seedlings are planted directly into the soil. Young plants are carefully shaded and irrigated after planting. The trees are also fertilised regularly with both organic and inorganic fertilisers. However, trees are rarely pruned and flowers are not hand-pollinated as they are in Thailand. On some farms, the trees are actively water-stressed so that off-season flowering is induced. The farmer can then receive a premium price for off-season fruit.

Intercropping is a common practice among Vietnamese durian growers. Longan, papaya, coffee and langsat are planted during the establishment of the durian orchard, both to provide shade and to provide additional income in the years before the durian trees bear fruit. Durian is increasingly intercropped with rice in the lowlands of the Mekong Delta in the early stages of orchard establishment.

Australia

In 1999–2000, an industry census identified approximately 12,000 grafted durian trees in the Darwin region of the Northern Territory and northern Queensland (Tully to Cape Tribulation) in Australia, but none in the tropical north of Western Australia (Zappala and Zappala 1994; Zappala et al. 2002). The identification of clones with greater tolerance to cool temperatures would be required for the area of production to expand any further south along the Queensland coast (Zappala et al. 2002). A vigorous Australian industry has the potential to fill seasonal production gaps in Southeast Asia between January and April, but as plantings are yet to reach maturity, annual production is currently less than 50 t (Zappala et al. 2002).

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8.2 Occurrence, Distribution and Utilisation of Durian Germplasm

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Abstract

Durian is a domesticated Asian rainforest tree that has been selected for fruit quality and yield. A few genotypes now dominate commercial cultivation. This narrow genetic base limits the expansion of durian cultivation, and exposes a serious vulnerability to pests and diseases in these new environments. The remaining natural diversity of durian genotypes is threatened by habitat destruction. Naturally occurring disease resistance is one key aspect of this diversity that remains to be fully exploited, in part due to the lack of reliable bioassays. This chapter catalogues the diversity of the genus and assesses the potential for new cultivars.

Introduction

Although it is commonly believed that *Durio* spp. evolved in Peninsular Malaysia, Borneo and Sumatra, durian (*Durio zibethinus* L.) is commercially grown as far west as Madagascar and India to Papua New Guinea in the east (Kostermans 1958; Subhadrabandhu and Ketsa 2001; Figure 8.2.1). Of the 30 recorded species (Table 8.2.1), 19 are found on the island of Borneo (total of Sabah, Sarawak and Kalimantan in Table 8.2.1), 16 on Peninsular Malaysia, and eight on Sumatra.

Durio zibethinus is the only species cultivated on a large scale commercially, but since this species is open-pollinated, it includes considerable diversity in fruit colour, aril size, seed size and tree phenology (Figure 8.2.2). A further eight species yield edible fruit (Tinggal et al. 1994; Voon Boon Hoe 1994):

- *D. graveolens* Becc., ‘durian burung’, ‘durian kuning’, ‘durian merah’, ‘tabelak’ or red-fleshed durian, has sweet, crimson-coloured arils and a fragrance of roasted almonds (Figure 8.2.3)
- ‘durian suluk’ is probably a natural hybrid between *D. zibethinus* and *D. graveolens*, and retains the flavour and texture of *D. zibethinus* with subtle burnt caramel overtones reminiscent of *D. graveolens*



Figure 8.2.1 Distribution of *Durio* species: solid line represents the native occurrence, while the dashed line represents the current extent of commercial production.

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Table 8.2.1 Distribution of *Durio* spp.

Species	Pen. Mal. ^a	Sabah	Sarawak	Kalimantan	Sumatra	Java-Java	Myanmar	Celebes	Philippines	Thailand	New Guinea	Indo-China
<i>D. acutifolius</i>	+	+	+	+								
<i>D. affinis</i>		+	+	+								
<i>D. beccarianus</i>												
<i>D. carinatus</i>	+		+		+							
<i>D. crassipes</i>		+	+									
<i>D. dulcis</i>		+	+	+					+			
<i>D. excelsus</i>			+	+								
<i>D. grandiflorus</i>	+	+	+						+			
<i>D. graveolens</i>	+	+	+	+	+				+			
<i>D. griffithii</i>	+	+	+	+	+							
<i>D. johoricus</i>	+											
<i>D. kinabaluensis</i>		+										
<i>D. kutejensis</i>		+	+	+		+			+			
<i>D. lanceolatus</i>		+	+	+								
<i>D. lissocarpus</i>			+	+								
<i>D. lowianus</i>	+				+							
<i>D. macrantha</i>					+							
<i>D. macrolepis</i>	+											
<i>D. macrophyllus</i>	+											
<i>D. malaccensis</i>	+				+							
<i>D. mansoni</i>							+					
<i>D. oblongus</i>			+									
<i>D. oxleyanus</i>	+	+	+	+	+				+			
<i>D. perakensis</i>	+											
<i>D. pinangianus</i>	+											
<i>D. purpureus</i>				+								
<i>D. singaporensis</i>	+											
<i>D. testudinarium</i>		+	+	+								
<i>D. wiyatt-smithii</i>	+											
<i>D. zibethinus</i>	+	+	+	+	+	+	+	+	+	+	+	+

^a Pen. Mal. = Peninsular Malaysia

- 'durian simpor' is a mild-flavoured, yellow-fleshed variant of *D. graveolens*
- *D. testudinarius* Becc. (syn. *D. macrophyllus* Ridley), the 'tortoise' or 'kura kura' durian, is a self-pollinated species, and thus less variable, that has an extended flowering season. The fruit ripens from green to yellow and the aril is pale yellow, sweet and has a strong aroma (Figure 8.2.4)
- *D. oxleyanus* Griff., 'durian sukan', 'durian beludu', 'isu' or 'kerontangan', is a very tall tree that produces small, round, green fruit adorned with long spines. The aril is yellow, smooth-textured and sweet (Figure 8.2.5)
- *D. kutejensis* (Hassk.)Becc., 'durian pulu', 'durian merah', 'nyekak' or 'lai', is a species that bears fruit late in the season. The flowers emit a strong carrion smell at anthesis, and the fruit has thick, golden arils with a mild, sweet taste and creamy texture (Figure 8.2.6)
- *D. dulcis* Becc., 'durian marangang', the red, 'tutong', or 'lahong' durian, produces fruit with attractive long red spines, and although the aril surrounding the shiny black seeds is thin, it has a sweet flavour and pleasant turpentine odour (Figure 8.2.7)
- *D. lowianus* Scort. Ex King, 'durian duan' has red flowers and elongated, oval-shape fruit containing white to yellow arils (Figure 8.2.8).

Numerous cultivars of durian have arisen in Southeast Asia over hundreds of years of selection from open-pollinated seedlings for fruit quality and yield (Lim and Luders 1997). The following attributes are more recently sought in current germplasm assessment schemes (Lim and Luders 1997):

- aril recovery of $\geq 30\%$
- yellow to deep yellow, firm, creamy aril
- small seed
- high (70 to 100 fruit per tree) and consistent yield
- resistance to major pests and diseases.

Historically, durian used to be grown from seeds with superior taste and texture but at present cultivars are propagated by either layering, marcotting or, more commonly, by a variety of grafting methods, including bud, veneer, wedge, whip or U-grafting onto seedlings of random rootstocks (Chapter 8.3; Lim and Luders 1997). In Thailand, the *D. zibethinus* cultivar Chanee is the preferred rootstock due to its observed resistance to infection by *Phytophthora palmivora*. Many superior selections have been identified in Malaysia through competitions held at the annual Malaysian Agriculture, Horticulture and Agrotourism Shows. The use of durian competitions to identify superior varieties and promoting extension has also being adopted in Vietnam by the Southern Fruit Research Institute (Figure 8.2.9).



Figure 8.2.2 Fruit diversity in *Durio zibethinus*.



Figure 8.2.3 Fruit of *Durio graveolens*.



Figure 8.2.4 Fruiting tree of *Durio testudinarum*, with inset showing internal view of the fruit.

More than 200 varieties of *Durio zibethinus* are recognised in Thailand and most originate from seedlings of open-pollinated fruits, however there are often only minor differences between varieties (Tinggal et al. 1994). There are many variations in the

spelling of Thai durian cultivars. For consistency, we have used the same spelling as Nanthachai (1994). Table 8.2.2 provides some of the alternative spellings that we have encountered for the most common varieties. Where Thai varieties have been introduced into other countries, there are yet more spelling variations, e.g. in the Philippines, Monthong is called Otong and Chanee is called Kani. Many attempts have been made to group the varieties according to either: (i) time to fruit-bearing from planting; (ii) fruit characteristics and origin of the variety; or (iii) length of time to fruit maturity (Tinggal et al. 1994; Lim and Luders 1997).

Hiranpradit and colleagues in 1992 proposed the following six groups by classifying varieties on leaf and fruit spine characteristics and fruit shape (Tinggal et al. 1994; Lim and Luders 1997):

- Kob – containing 38 varieties
- Luang – containing 7 varieties, including Chanee
- Kanyao – containing 7 varieties, including Kanyao
- Kumpun – containing 11 varieties, including Monthong
- Tongyoi – containing 12 varieties
- Miscellaneous – containing 47 varieties including Kradoom.

Tinggal et al. (1994) present photographs of fruit representative of each group, while Lim and Luders (1997) give detailed descriptions.

Despite the large number of varieties, the area under cultivation in Thailand's world-leading export industry is dominated by just four varieties: 41% Monthong, 33% Chanee, while Kanyao and Kradoom represent about 8.5% of the cultivated area (Alim et al. 1994; Zappala 2002). Thai varieties have been introduced to many other durian-producing countries and Monthong and Chanee are recommended varieties in Malaysia and the Philippines.

Like Thailand, Malaysia has a multitude of open-pollinated varieties but only a small number are cultivated on a commercial basis. Two organisations in Malaysia have hybridisation programs using

Table 8.2.2 Alternative spellings of common Thai durian varieties.

Variety name	Alternative spellings			
Kob	Kop	Gob		
Luang	Lueng			
Kanyao	Gaan Yao(w)	Karn-Yao	Kan Yau	
Kumpun	Kampun	Gumpun		
Monthong	Montong	Mon Thong		
Kradoom	Kadoom	Kradum Thong	Kra-dum-tong	Gradumtong

popular local varieties as parents — the Malaysian Department of Agriculture (MDA) and the Malaysian Agriculture Research and Development Institute (MARDI) (Lim and Luders 1997). The MDA program started in the 1960s, registration of hybrids occurred in the 1980s and the first reports of commercial success with the hybrids came in the early 1990s (Brown 1997; Lim and Luders 1997), demonstrating the long-term investment required for breeding programs. Lim and Luders (1997) describe the origins and fruit characteristics of over 100 Malaysian varieties, including the ones recommended for cultivation by MDA. MARDI now has one of the largest *Durio* germplasm collections in the world, containing approximately 400 accessions (Brown 1997).

Lim and Luders (1997) also describe over 40 of the recognised Indonesian varieties, including the 15 superior varieties that have been released and recommended by the Indonesian Department of Agriculture. Tinggal et al. (1994) describe the six cultivars recommended for planting in the Philippines, the three varieties grown in Singapore and some of the other *Durio* species cultivated for local consumption in Brunei, including *D. graveolens*,

D. testudinarum, *D. oxleyanus*, *D. kutejensis*, *D. dulcis* and 'durian suluk'.

There is very little detailed information readily available on the commercial varieties available in countries like Cambodia, Laos, Myanmar (Burma), Sri Lanka and Vietnam (Lim and Luders 1997). However, Vietnamese local selections are numerous and show large variations in yield, fruit quality and disease susceptibility. The area of durian under cultivation is expanding rapidly in southern Vietnam, generating significant new wealth and improving living standards for farmers.

All the varieties currently found in Australia have been introduced from Southeast Asia. In contrast to other durian-producing countries where industry development has been strongly promoted by government, the effort to establish a viable industry in Australia has been driven mostly by enthusiastic farmers (Lim and Luders 1997). Durian production in northern Queensland is a relatively new industry with approximately 9000 trees grown from Cooktown (16°S) to Tully (18.5°S) along the wet tropical coast.

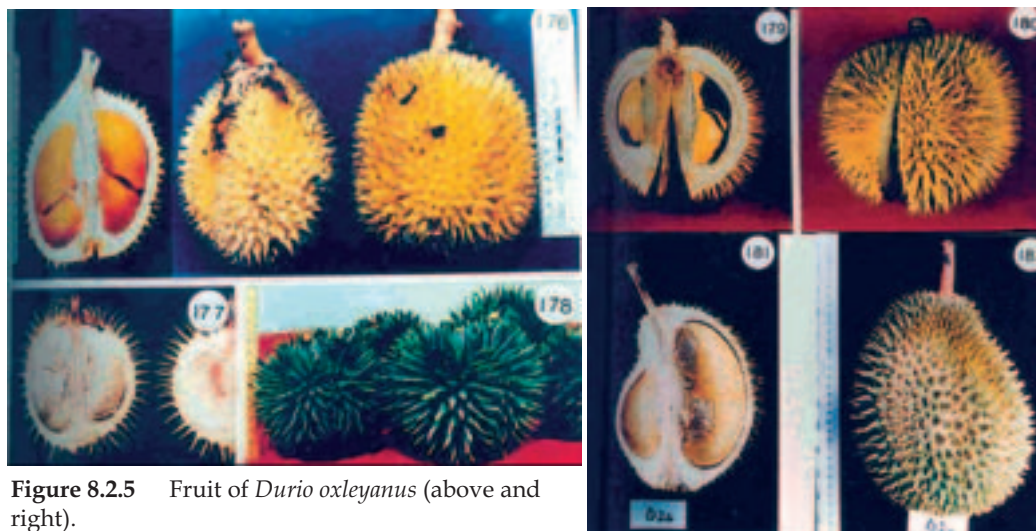


Figure 8.2.5 Fruit of *Durio oxleyanus* (above and right).



Figure 8.2.6 Fruit of *Durio kutejensis*.

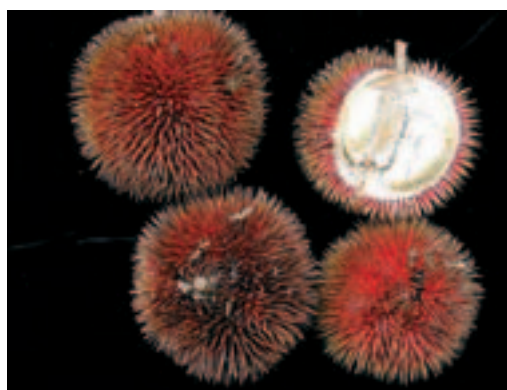


Figure 8.2.7 Fruit of *Durio dulcis*.

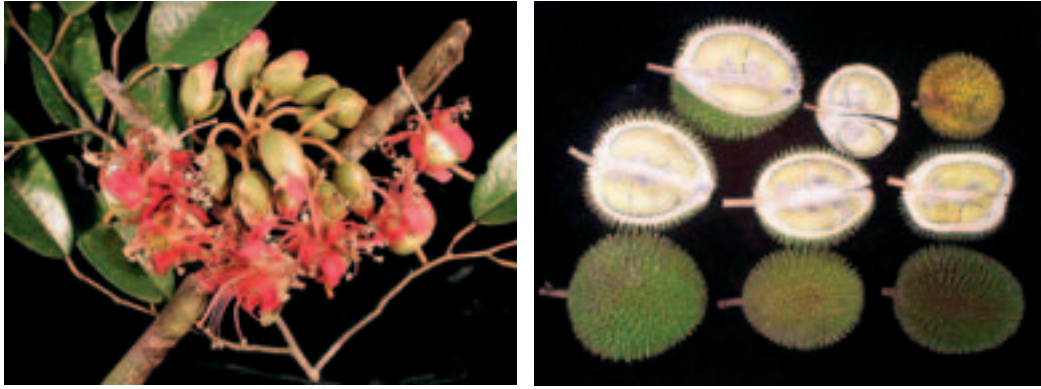


Figure 8.2.8 Flowers and fruit of *Durio lowianus*.

Durian seeds were first imported into Australia in the early 1970s from Malaysia, Indonesia and Thailand (Watson 1984). As growers gained a taste for, and a commercial interest in, durian, budwood and grafted trees were imported. Approximately 40 clones of *Durio zibethinus* and seven other *Durio* species have been introduced into Australia, including *D. dulcis*, *D. kutejensis*, *D. oblongus*, *D. oxleyanus*, *D. testudinarum*, *D. macrantha* and *D. graveolens* (Lim 1998). In addition, over 50 cultivars of *D. zibethinus* and 30 clones from guaranteed sources in Malaysia, Thailand and Indonesia were evaluated for suitability to Australian conditions (Zappala 2002).



Figure 8.2.9 Durian competition at the Southern Fruit Research Institute, Vietnam.

Varieties that are showing promise and being grown in commercial orchards include Monthong (Thailand), Luang (Thailand), D24 (Malaysia), D2 (Malaysia), Hew 2 and 7 (Malaysia), Hepe and Permasuri (Indonesia). A number of local seedling selections have been made and include Limberlost and Chong. Several other *D. zibethinus* clones (D 175, DPI Monthong, Hawaiian Monthong, D190 and Kradum Thong) and *D. macrantha* should also be considered for commercial production in northern Queensland (Zappala 2002).

Some of the durian material introduced in the 1970s and 1980s did not exhibit true varietal characteristics, and recent DNA fingerprinting has confirmed their initial misidentification (Zappala et al. 2002). Misidentification of the germplasm has been a major constraint to the establishment of a successful and credible industry in Australia.

Genetic Erosion of *Durio* Germplasm

Brown (1997) expressed concern about the genetic erosion of *Durio*. Despite what seems like a lot of variety within *D. zibethinus*, the trend in Indonesia, Malaysia, Thailand and Vietnam toward cultivating clonal material of a few popular commercial varieties is interpreted as contributing to this genetic attrition. Furthermore, there is great scope for improvement and further development of durian cultivars. The ideal tree would be small to facilitate management and harvesting, would be precocious and have a long bearing season, and would bear fruit with a mild odour, large arils and good flavour. The tree would also be environmentally tolerant and resistant to the major diseases and pests.

There are many known species that have not yet been fully described, and the existence in wild populations of other species with edible fruit, resistance to pathogens and other desirable agronomic characteristics remains unexplored. For example, *D. lowianus*, a wild durian from southern Thailand, is apparently more resistant to *P. palmivora* than many commercial cultivars. However, massive deforestation in the centre of diversity of *Durio* seriously threatens the survival of this diversity, and some wild species are probably already extinct.

Scientists must preserve genetic diversity for use in breeding programs. Current germplasm collections should be supplemented by the preservation of large tracts of forest in which wild species are growing, as the genetic conservation of *Durio* using conventional methods is limited because:

- collection sites are limited because durian cultivation is restricted to the humid tropics
- durian is either unknown or not highly regarded outside Southeast Asia
- seeds have a short period of viability and thus conservation in a conventional seedbank is unsuitable
- cryopreservation of seed and callus is still being investigated but is not yet reliable
- attempts to regenerate durian callus have so far been unsuccessful
- trees are very large, making a 'living germplasm' collection impractical and costly for the maintenance of a large numbers of accessions
- germplasm collections kept in high density and in suboptimal environmental conditions can be severely affected by pests and disease
- in order to maintain the diversity present in open-pollinated varieties, a significant number of trees needs to be maintained on an on-going basis.

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8.3 Screening for Resistance to *Phytophthora*

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Abstract

Identifying and evaluating disease resistance depends on rapid, reliable and robust bioassays that can rapidly screen large numbers of genotypes and breeding progenies. We developed seedling, leaf and stem bioassays to screen durian germplasm from Thailand, Vietnam and Australia for resistance to *Phytophthora palmivora*. Detached leaf assays segregated durian cultivars into classes consistent with field observations, and are recommended as an early screen in breeding programs. Durian cultivar Chaneé emerged as the least susceptible cultivar in Thai and Vietnamese tests.

Screening Germplasm for Tolerance to *Phytophthora*

Disease-resistant varieties are central to the integrated management of *Phytophthora palmivora* in durian. Lim (1998a) suggested that wild *Durio* spp. evolving in damp, low-lying areas may be potential sources of genes for disease resistance against *Phytophthora*. The relatively few resistance studies reported suggest that resistance in durian is polygenic (Lim 1998b). One of the major aims of Australian Centre for International Agricultural Research (ACIAR) Project PHT/1995/134, ‘Management of *Phytophthora* diseases in durian’, was to develop a rapid and reliable resistance screening bioassay to identify sources of resistance

in the germplasm collections of Thailand, Vietnam and Australia.

The resistance screening of a perennial crop such as durian might involve pot trials in which whole plants are artificially inoculated, or field trials in which trees at infested sites are assessed over time for disease development and survival. These tests are time-consuming and expensive, and considerable savings could be made if more rapid assays enabled more cultivars to be screened. Preliminary screening bioassays designed to identify cultivars with promising disease-resistance characteristics, or with high levels of susceptibility, have been successfully developed for other crops using detached plant organs.

One of the major diseases of cocoa is black pod, caused by *Phytophthora* spp., and screening bioassays have been developed using detached whole leaves, leaf-discs (Nyasse et al. 1995) and detached cocoa pods (Iwaro et al. 1997). Such bioassays have been used to expedite the identification of resistant genotypes that are suitable for cocoa breeding programs, or susceptible genotypes that should be excluded. Cocoa typically produces two pod flushes a year, with the main cropping season lasting up to six months. With such long production cycles, cocoa pods can be available for screening experiments most of the year. The distinct and relatively short fruiting period of durian makes fruit bioassays less practical as a

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routine tool. Additionally, the large size and the high value of durian fruit can make the design of statistically valid screening experiments difficult.

The variation in the pathogen population means that testing of cultivars at more than one place is necessary. At present, it is unclear if different pathogenic races or differences in aggressiveness occur among *P. palmivora* populations in Southeast Asia and Australia. In addition to differences in pathogen populations, we also have to consider differences in environmental conditions and soil types which occur at a local level and may have a significant influence on the expression of resistance in durian cultivars.

Bioassay Development

Entire leaf versus leaf-strip

Some durian cultivars have very large leaves, making the use of entire leaves in a bioassay unwieldy. Leaf-strips (approximately 6 cm long by 2.5 cm wide) cut from either side of the main vein can be used as an alternative. Although we found no difference in the rate or magnitude of lesion development between entire leaves and leaf-strips, there were disadvantages using leaf-strips. Fungal contamination at the cut edge of the leaf-strip was common, particularly if the leaves had been sourced from an orchard rather than from glasshouse-grown seedlings. We reduced contamination by surface-sterilising leaf-strips in a mixture of 10% ethanol and 3% a.i. sodium hypochlorite for 1 minute, followed by thorough rinsing in sterile deionised water before inoculation. However, the production and surface sterilisation of individual strips makes this a time-consuming process.

Wounded versus non-wounded leaf material

Ideally a bioassay includes wounded and non-wounded treatments so that tissue susceptibility to penetration and infection can be assessed independently. However, in bioassay experiments in Australia (Tan 1999) and Thailand, non-wounded durian leaves did not develop disease symptoms reliably when inoculated with *P. palmivora*. Consequently, a wounding device was designed to deliver a consistent wound to leaves (Figure 8.3.1) before inoculation with an agar plug from the edge of a colony of *P. palmivora*.

Incubation conditions

Where ambient temperatures were too cold or variable for infection to occur, incubation was carried out in constant-temperature cabinets at 26°C. Tissue desiccation was successfully avoided by incubating whole detached leaves on wire mesh

platforms over free water, in sealed Tupperware® containers. However, incubating leaf-strips over free water, as described above, did not prevent desiccation. While desiccation was reduced by laying the leaf-strips on paper-towel moistened with sterile water, cross-contamination was common due to accidental contact between the leaf-strips, or colonisation of the towel by the pathogen. Tissue desiccation and cross-contamination were prevented when leaf-strips were inoculated at one end and the non-inoculated ends were placed vertically into slots made in a layer (75 mm deep) of solidified water agar and incubated in a sealed Tupperware® container (Figure 8.3.2). Although more time-consuming, an additional advantage of placing the strips vertically rather than horizontally was that many more strips could be accommodated in a single tray, increasing the number of samples that could be tested in a single bioassay.



Figure 8.3.1 Wounding device, constructed from a clothes peg and thumb-tack, designed to standardise the wounding of leaves.



Figure 8.3.2 Inoculated durian leaf-strips standing vertically in water agar to keep them turgid during incubation.

Symptom assessment in leaves

Depending on incubation conditions, it may take up to three days from inoculation to the appearance of the first disease symptoms. Measurement commences as soon as symptoms appear. When entire leaves are inoculated, lesion diameter is measured. As lesions are often not concentric, it is recommended that the diameter be measured in more than one direction, then averaged. In leaf-strips, the length of the lesion from the wound to the leading edge of the lesion should be measured.

Stem bioassay

Detached-stem bioassays are better for comparing clonal lines of *Eucalyptus marginata* for susceptibility to *Phytophthora cinnamomi* (Hüberli 2002) than for comparing pathogenicity between isolates of the pathogen (Hüberli 2001). Durian stems are readily available, can be obtained from large trees without undue injury, and as such should be suitable for use in a bioassay. However, attempts to develop a bioassay for durian using detached stems were unsuccessful.

Green stems (stems in which periderm formation had not yet occurred), with diameters 0.50–1.25 cm, were obtained from durian orchards in northern Australia. Each stem was cut to a length of 15 cm before surface sterilisation for 2 minutes in the solution described above. The holes in non-draining test-tube racks were half filled with washed/sieved sand and 2 mL water that contained 50 µg/mL benzimidazole. The rack was autoclaved and a stem placed upright into each of the holes. A plug of inoculum mycelium/sporangia was placed onto the end of each stem and the rack was then put into a Tupperware® container and sealed for incubation.

Despite a more rigorous surface sterilisation, the stems were rapidly colonised by secondary invaders. Unlike *E. marginata*, lesions were not visible from the outside of the inoculated durian stem. Even when the epidermis was scraped away, it was difficult to see the lesions, and, if visible, to determine the lesion boundary. When the stems were split longitudinally, the pith often appeared orange but this may have been due to oxidation of the exposed tissues. Due to the difficulty of definitively identifying and measuring lesions, the stem was dissected into 1 cm segments, which were plated sequentially onto selective agar to calculate how much of the tissue was colonised by the pathogen. A bioassay using excised stems as described above is time-consuming, expensive and consequently considered unsuitable as a rapid and inexpensive screen for resistance in durian.

In summary, leaves are the most practicable durian organ to use in a detached-organ screening bioassay. Where incubation space is not limiting, the use of entire leaves is recommended due to the labour-intensiveness of producing strips or discs. Where incubation space is limiting, leaf-strips or discs can be used but surface sterilisation must be rigorous to minimise contamination and interference by secondary pathogens.

Germplasm Screening in Thailand

Field observations in Thailand indicate that durian cultivar Chanee is moderately resistant to infection by *P. palmivora*, while Kadoom, Kanyao and Monthong are susceptible. The four cultivars were screened in controlled experiments using the following methods:

- attached leaves, wound inoculated with mycelium/sporangia
- attached stem, wound inoculated with mycelium/sporangia
- detached fruit, wound inoculated with mycelium/sporangia
- attached unwounded root, inoculated with a sporangial suspension for five days
- measurement of zoospore production from sporangial suspension into which seedling roots were immersed (Figure 8.3.3).



Figure 8.3.3 Germinated durian seeds immersed in a sporangial suspension.

Controls were inoculated with sterile agar or water. Percentage disease incidence was measured in leaf, stem and fruit by estimating the amount of the tissue covered by lesions. In roots, disease incidence was calculated by plating sequential segments of the roots onto selective media and calculating the number of pieces from which the pathogen grew. Additionally, colonisation of the root was assessed through examination under a dissecting microscope,

looking for mycelium and sporangia and expressed as a percentage of the root examined.

Symptoms were similar for all cultivars in that lesions produced on leaves were dark brown, and on fruit were light brown and soft. Lesions did not develop at the point of inoculation on stems, rather the terminal part of the inoculated branch wilted and leaves abscised.

The disease incidence in the screening bioassays agrees with the field performance of cultivar Chanee. Leaf, stem, fruit and root tissues were less susceptible than Kadoom, Kanyao or Monthong (Table 8.3.1). Similarly, *P. palmivora* colonised significantly fewer Chanee roots, and produced fewer zoospores.

Germplasm Screening in Vietnam

A leaf-strip bioassay was performed on durian cultivars Chanee, D2, D6, D101, Goc Ghep, Hat Lep Dong Nai, Hat Lep Tien Giang, Kho Qua Xanh, La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre and Tu Quay. The cultivars were screened against three isolates of *P. palmivora* obtained from (i) soil, (ii) stem canker and (iii) leaf in diseased orchards of Tien Giang Province. Controls were inoculated with sterile agar. A second bioassay in which leaf-strips and detached stems were screened against the soil isolate was conducted on the same cultivars, with the replacement of cultivar Goc Ghep with Kho Qua V. Controls were inoculated with sterile agar. In both bioassays, lesions were measured five days after inoculation.

The soil isolate was more virulent than either the canker or the leaf isolates, and in general the canker isolate was more virulent than the leaf isolate (Figure 8.3.4). Based on the symptoms produced by the virulent soil isolate, cultivars Tu Quy, Chanee and La Queo were less susceptible to the pathogen. The commercially popular Ri6 and Sue Hat Lep Ben Tre emerged as two of the most susceptible cultivars.

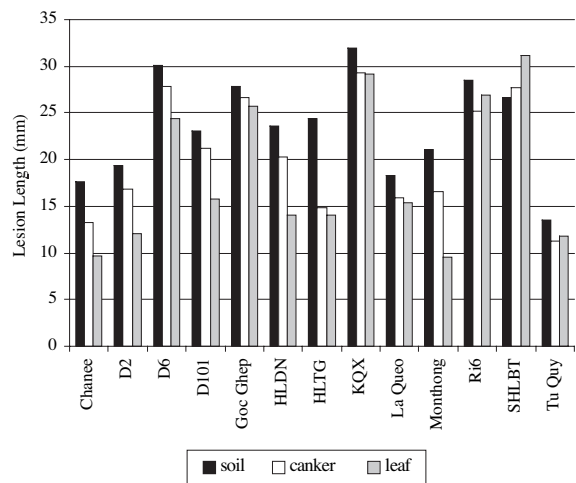


Figure 8.3.4 The length of lesions (mm) on leaf strips of durian cultivars Chanee, D2, D6, D101, Goc Ghep, Hat Lep Dong Nai (HLDN), Hat Lep Tien Giang (HLTG), Kho Qua Xanh (KQX), La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre (SHLBT) and Tu Quay, five days after inoculation with isolates of *Phytophthora palmivora* from either soil, canker or leaf. Controls were inoculated with axenic agar.

Table 8.3.1 Disease incidence (%) in attached leaves, attached stems and detached fruits of durian cultivars Chanee, Kanyao, Kadoom and Monthong inoculated with *Phytophthora palmivora*, as well as disease incidence and colonisation of the roots, and zoospore production (time in minutes to zoospore release and numbers of zoospores) in 0.5 mL sporangial suspension in the presence of roots.

Durian cultivars	Field observations	Attached leaves	Attached stems	Detached fruit	Attached roots		Zoospore production	
					Incidence	Colonisation	Number of zoospores	Time to zoospores
Chanee	MR	47a	20a	20a	10a	60a	76a	45
Kanyao	S	100b	100b	100b	45b	100b	119b	30
Kadoom	S	100b	100b	100b	50b	100b	124b	15
Monthong	S	100b	100b	100b	50b	100b	190c	15

Note: means followed by the same letter are not significant difference at the 5% level by Duncan's multiple range test (DMRT); MR = moderately resistant, S = susceptible.

In the second bioassay, pathogen growth in the detached stems was limited (Figure 8.3.5). However, taken together with the results of the first screening, results from the detached leaves indicate that Tu Quy and Chanee may be suitable for use as rootstocks, while Ri6 is inappropriate because of its susceptibility (Figure 8.3.5).

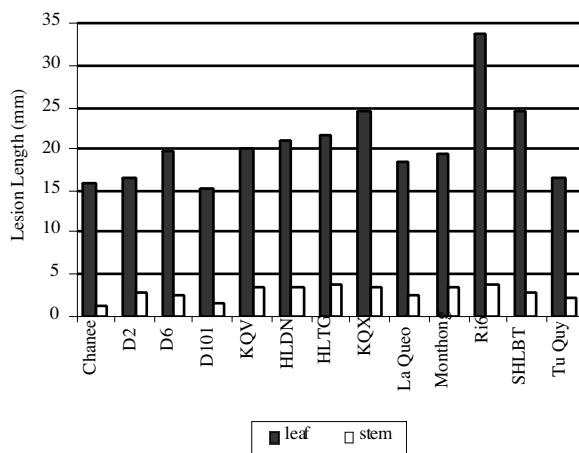


Figure 8.3.5 The length of lesions (mm) on leaf strips and detached stems of durian cultivars Chanee, D2, D6, D101, Kho Qua V (KQV), Hat Lep Dong Nai (HLDN), Hat Lep Tien Giang (HLTG), Kho Qua Xanh (KQX), La Queo, Monthong, Ri6, Sua Hat Lep Ben Tre (SHLBT) and Tu Quay, five days after inoculation with an isolate of *Phytophthora palmivora* soil. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph.

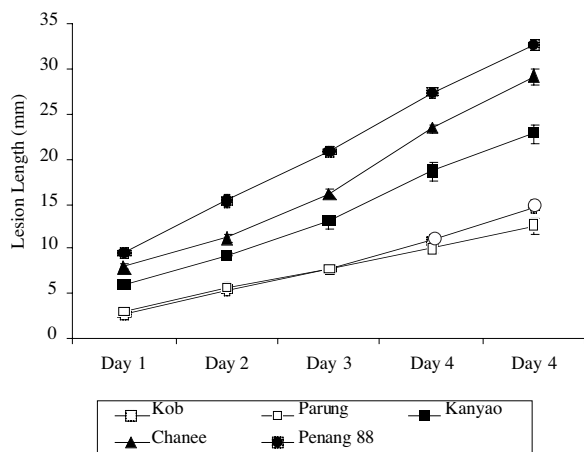


Figure 8.3.6 Mean lesion diameter (mm) in durian (*Durio zibethinus*) cultivars Chanee, Kanyao, Kob, Parung and Penang 88 in a detached-leaf bioassay. The remaining 14 cultivars screened – Chompoosee, D10, D24, D98, D102, D123, Kobyao, Kumpun, Hew 3, Kradoom, Luang, Limberlost, Red Prawn and Sunai – and *Durio macrantha* fell between Kanyao and Penang 88. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph. Vertical bars are standard errors of the means.

Germplasm Screening in Australia

In summer 2000/2001 at the Centre for Wet Tropics Agriculture, *Durio macrantha* and 19 cultivars of *D. zibethinus* were screened in a detached-leaf bioassay against a locally obtained trunk-canker isolate of *P. palmivora*. The durian cultivars screened were Chanee, Chompoosee, D10, D24, D98, D102, D123, Kanyao, Kob, Kob Yao, Kumpun, Hew 3, Kradoom, Luang, Limberlost, Parung, Penang 88, Red Prawn and Sunai. Controls were inoculated with sterile agar. In autumn 2002, Chanee, D10, Kob, Hew 3 and Monthong were screened against the canker isolate and a root isolate, as well as a fruit isolate which showed low virulence in preliminary trials (Tan 1999). Controls were inoculated with sterile agar. In both bioassays, lesion extension was measured daily from two to six days after inoculation. The summer screening indicated that Kob and Parung were less susceptible to infection by *P. palmivora* than the other cultivars (Figure 8.3.6). The ranking of isolates that were screened twice was the same for the summer and autumn bioassays, from Kob, the least susceptible cultivar, to D10, the most susceptible cultivar, with Hew 3 and Chanee displaying intermediate susceptibility. In the autumn screening, the largest lesions were produced in Monthong, which is in agreement with published and anecdotal evidence stating that it is highly susceptible. The fruit isolate caused significantly smaller lesions than either the canker or root isolates (Figure 8.3.7), confirming the results of Tan (1999).

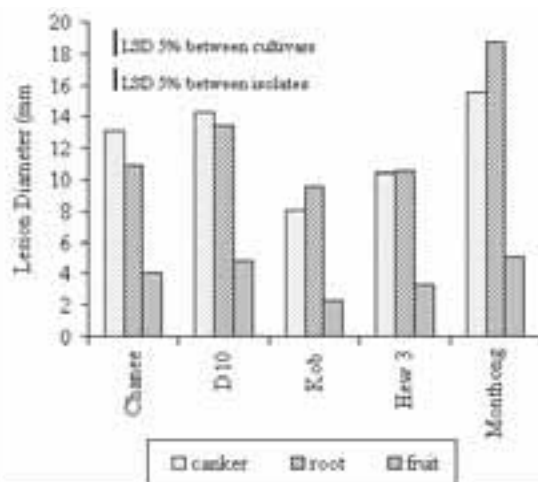


Figure 8.3.7 Mean lesion diameter (mm) in durian cultivars Chanee, D10, Kob, Hew 3 and Monthong in a detached-leaf bioassay with isolates of *Phytophthora palmivora* from either canker, root or fruit. Controls, which were inoculated with axenic agar, did not develop lesions and are not shown in this graph. Vertical lines represent the least significant difference (LSD).

Chanee emerged as one of the most susceptible cultivars tested (Figure 8.3.6 and 8.3.7) in Australia, which contradicts the experimental evidence from Thailand and Vietnam. Cultivar D10 also developed extensive lesions indicating high susceptibility, which is in contrast with previous reports (Lim 1998b). As discussed earlier in this paper, these discrepancies could arise from pathogen differences between Australia and Thailand, or due to erroneous identification and labelling of durian germplasm imported into Australia in the 1970s and 1980s (Lim 1998a). DNA testing confirmed that the originally introduced Chanee had been misidentified on introduction to Australia (Zappala et al. 2002).

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8.4 Durian Propagation and Nursery Practice

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Abstract

This paper details nursery best practice procedures to ensure the supply of adequate quantities of vigorous, disease-free seedlings to the durian industry. Procedures adopted in Vietnam and Australia are compared and contrasted.

Introduction

Best practice in durian nurseries is fundamental to the establishment of healthy durian orchards. In Vietnam, the durian industry is rapidly expanding, but there is a general shortage of selected durian cultivars. As has been seen in other rapidly expanding horticultural industries, high demand for planting material can lead to shortcuts being taken in nursery practice, resulting in poor-quality and variable planting material. This can be a serious problem when soil-borne pathogens such as *Phytophthora* species, are spread from infected nursery stock to newly established orchards. As a consequence, what may have been a disease-free orchard becomes infested. Once established, pathogens like *Phytophthora* are practically impossible to eradicate. In established durian-growing countries, such as Thailand, nursery operators have developed considerable expertise in propagating selected cultivars for distribution to orchards. However, even here, soil-borne disease can be a problem if nursery hygiene is not carefully

implemented and monitored. The impacts of diseases like phytophthora on nurseries include the direct costs due to plant deaths, and the difficulties and extra costs associated with managing diseases, poor-plant quality and damage to the nursery's reputation among customers.

Propagation Techniques

Nurseries use a range of propagation techniques to service the rapidly expanding durian industries in Vietnam. The particular technique favoured depends on the availability of selected genotype stock and scion material, the quantity of planting material required, the price paid by purchasers, and labour costs and skills.

Cho Lach District in Ben Tre Province in the Mekong Delta of Vietnam is well known for its production of fruit tree saplings. The Cho Lach people learnt grafting techniques from the French around 100 years ago and now produce more than 20 million citrus, durian, mango, longan, mangosteen and rambutan saplings annually. A hard-working family in this area can produce 30,000 to 40,000 durian plants each year. In general, the quality of the nursery stock is good, as the nurserymen and women are skilled and experienced.

In Australia, durian planting material is provided by a small number of nurseries where the proprietors are usually also durian growers. The Australian durian industry is relatively small and still in its infancy, hence clonal production is based on a range of cultivars as part of longer-term, regional cultivar

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testing. In the past, seed supply was limited, and imported seed, mainly of Indonesian and Malaysian origin, was the main source of seedling stock. Australian-grown fruit not suitable for fresh fruit sales were also keenly sought as a source of seed. The genetic base of rootstock is extremely variable and may explain some of the differences in tree performance and survival seen in the field. Seeds were sown either in bulk or into individual pots (2–5 L plastic bags; Figure 8.4.1). Potting mix varies between nurseries, but generally consists of a mixture of sand, soil and composted organic matter (pine bark, peanut shells or similar). In some cases, vermiculite or perlite is used in place of organic compost. Some growers have found that a more open (aerated) mixture results in improved root growth and seedling vigour (Figure 8.4.2). One major producer of durian planting material has moved to a soil-less mix consisting of 80% composted pine bark and 20% sand (Zappala et al. 2002). Potting mix is rarely pasteurised at present, but is being considered against a background of improved understanding of how disease is transferred.

A major innovation has been the introduction of raised nursery benches, which allow pots to be placed above the ground, hence minimising contamination of new pots and plants by water movement on the nursery floor.

Propagation techniques are evolving as nurseries learn and develop new and more reliable techniques. Nurseries have used approach grafting, marcotting, budding and wedge-graft techniques. Bud grafting utilising the Fokert technique was initially the preferred method of propagation. In the

Northern Territory, Lim (1997) reported that cleft-grafting techniques were as successful as Fokert budding, but the time of year was crucial to maximal success. Zappala et al. (2002) also presented data that confirm that propagation during the warm, wet season resulted in higher success (generally greater than 60%) than propagation carried out under cool, dry conditions.

Australian nurseries, like their Vietnamese counterparts, now predominately use a wedge-grafting technique rather than Fokert budding. Actively growing, 6–12-month-old seedling material is preferred as rootstock. Scion material with one to two active buds is selected from healthy trees (Figure 8.4.3). One-third to one-half a leaf is left on the bud stick and the lower part of the stick is trimmed to a wedge shape. The stock stem is cut cleanly and split, and the bud stick is inserted and held together with plastic clothes pegs. The newly prepared graft is covered with a semi-opaque plastic bag and the pot placed in a warm, plastic house. The pegs are removed after a callus has formed 3–4 weeks after grafting (Figure 8.4.3). Some durian growers who produce planting material for their own use prefer to use an approach-graft technique (Figure 8.4.4).

In Vietnam, the traditional wedge-graft or budding technique was largely replaced by the U-grafting (side-graft) technique about six years ago. U-grafting allows four to five times the number of saplings to be produced per budwood (Figure 8.4.5). The U-grafting technique is also much easier to carry out than is traditional budding.



Figure 8.4.1 Durian seed germination



Figure 8.4.2 Well-aerated potting mix (80% composted pine bark:20% sand) results in greater root vigour (plant on right) relative to a plant grown in a soil mix.



Figure 8.4.3a (above) Scion material with one to two active buds is selected from healthy trees.



Figure 8.4.3c (right) New buds emerging from a wedge graft 3-4 weeks after grafting. Plastic clothes pegs are used to bind the grafts

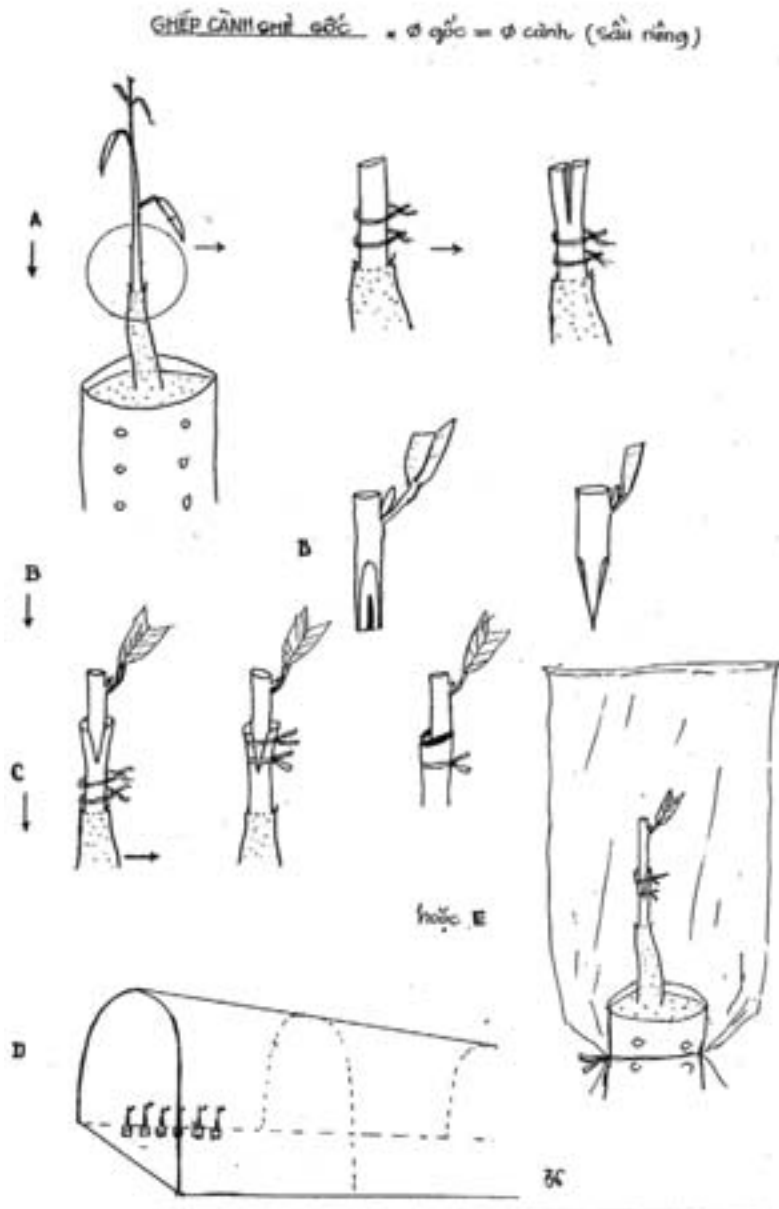


Figure 8.4.3b Wedge-grafting technique

Durian nurseries produce two types of durian saplings – one rootstock or two rootstocks. Saplings with two rootstocks establish and grow faster than single rootstock saplings. The wedge graft is used for two rootstock saplings, while U-grafts are used for single rootstock saplings. The time needed from sowing the seed to selling the plants is approximately 12 months.

As in Vietnam, double versus single rootstocks have been tested in Australia (Figure 8.4.4). Australian nurseries prefer to produce single rootstock material. Shortage of seedling stock, lower labour requirements and better long-term field survival of single-stock plants are the main reasons for preferring single rootstock material. Australian experience suggests that field survival of trees is

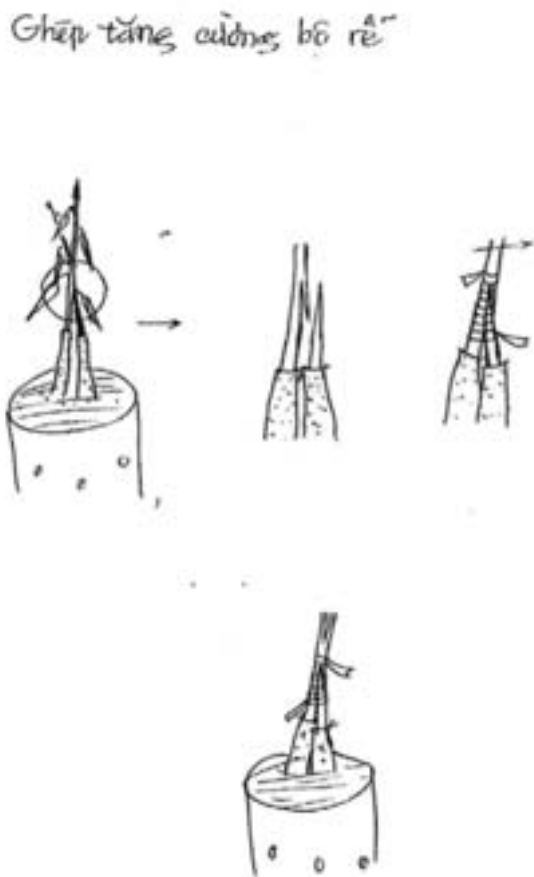


Figure 8.4.4b (above) Vietnamese durian approach-grafting technique.

Figure 8.4.4a (left) Approach graft used to create multiple rootstocks.



Figure 8.4.4c Approach grafting using plastic clothes pegs for graft clamping.



Figure 8.4.4d Advanced double rootstocks ready for planting (SOFRI, Vietnam).

enhanced if grafted trees are kept in the nursery until they have a trunk diameter of more than 12 mm and are approximately 1 m in height (Zappala et al. 2002). Australian nurseries have made little use of the side-graft technique, known in Vietnam as the U-graft. This method uses 12–24-month-old rootstocks, which in Vietnam are direct seeded into nursery beds and then uprooted and potted a month before grafting.

A few durian growers avoid using grafted planting material, preferring to use seedlings. Anecdotal evidence suggests that stock/scion incompatibility may affect the vigour and productivity of grafted durian. There are very few hard data on the

performance and disease susceptibility of durian stock/scion combinations, and this is an area in high need of further research.

Nursery Hygiene

It is important that more attention be paid to producing disease-free planting stock in the future, to prevent the spread of pests and pathogens. To achieve this, durian nursery operators need to follow best-practice methods, such as those established in the citrus and avocado industries and discussed in Chapter 7.2 (NGIA 2003). They also require access to reliable diagnostic services. Furthermore, it is advisable to accurately record and



Figure 8.4.5a Uprooted 18-month-old seedling being prepared for side or U-grafting (Vietnam)



Figure 8.4.5c Side-grafted durian seedling ready for planting

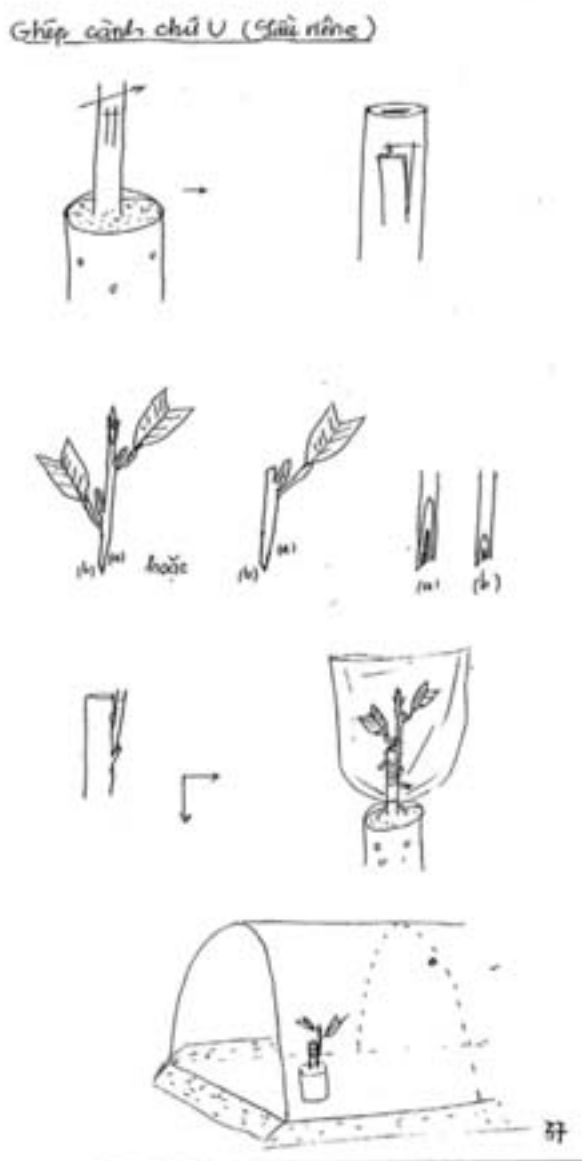


Figure 8.4.5b Side-grafting technique

regularly audit nursery procedures to ensure that recommended practices are being followed, and to identify difficulties. Ultimately, these procedures form the basis of a nursery accreditation scheme, guaranteeing high-quality, certified, disease-free planting material for growers.

The following best practices are recommended for durian nurseries:

- Nurseries should be established away from mature orchards on sites that are properly drained to avoid water entry or run-off.
- Only seed from disease-free fruit that has not been lying on the ground should be used to establish rootstocks.
- Only budwood from disease-free trees, taken from branches above the soil-splash level, should be used as scion material.
- Plant material from other nurseries should be quarantined in a separate facility and monitored for pests and diseases for at least four weeks.
- Potting media should be porous and free-draining. Soil, river sand or coconut fibre, should be avoided, as these substrates frequently contain *Phytophthora*, *Pythium*, *Rhizoctonia* and nematodes. Composts should be anaerobically fermented and matured for at least 10 weeks before use.
- All potting media should be thoroughly mixed on surfaces that are drained to exclude both water run-off and entry, and are free from soil and other sources of contamination.
- Potting media should be pasteurised by steam-air treatment.
- Pasteurised potting media should be stored in closed, disinfected containers, and must be regularly baited for *Phytophthora* before use.
- Potting media can be recycled, but must be steam-air pasteurised and stored hygienically.
- Nursery floors and paths should be sealed with concrete, or covered with coarse gravel at least 75 mm deep, and kept free of plant material and weeds.
- All pots, utensils, tools, containers and trolleys must be cleaned of soil or potting mix after use. Used pots and containers should be sterilised in 1% hypochlorite solution, and tools regularly disinfected with quaternary ammonium detergents (2000 ppm is recommended) or 70% methylated spirit. Hands must be washed with soap and water or an approved hand-washing biocide.
- Only pathogen-free irrigation water, preferably from deep bores, should be used. Irrigation water must be regularly monitored for pathogens, especially *Phytophthora*.
- Pots should be placed on raised, slatted benches and spaced to allow free air movement. Larger pots may be placed on raised beds of coarse gravel at least 75 mm deep, with adequate drainage to ensure that water does not accumulate or pond. In these cases, the gravel should be tested regularly and be certified pathogen-free.
- Watering hoses should be kept off the ground.
- Nursery areas should be fenced and secured to restrict access and prevent the entry of animals.
- Wind and dust should be suppressed.
- Plants should be grown in appropriate levels of light. Durian seedlings tolerate direct sunlight and overshadowing can cause disease problems.
- Appropriate fertiliser applications, preferably composted chicken manure, should be timed to ensure optimal nutrition and growth.
- Anyone entering the nursery area should wash their hands before entry, walk through a footbath containing copper fungicide, and not smoke or eat.
- Plants should be regularly inspected for pests and diseases and culled as required.
- Plants should be sold or distributed for planting before the roots become bound.
- Discarded plants and potting mix should be stored in designated closed containers and removed frequently. Discarded material may be anaerobically fermented and composted, or buried away from the nursery and drainage lines. Diseased plants should be burnt.
- Weeds in the pots and around the nursery beds must be rigorously controlled.
- Insect pests such as mealy bugs, aphids, thrips, white-fly, scale, mites and borers, should be managed, preferably using integrated pest management.
- Use of fungicides in the nursery should be avoided (especially phosphonates) as these may mask disease symptoms without eradicating the pathogen.

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8.5 Durian Tree Phenology and the Control of Phytophthora Diseases of Durian Using Phosphonate Trunk Injection

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Abstract

We have identified phenological patterns of mature durian trees grown in the north of Queensland, Australia, and monitored the distribution of phosphonate following trunk injection at three distinct phenological periods, to identify the injection period which results in maximum uptake in all tree organs. Durian cultivars Gumpun, Parung and Gob Yaow were injected with 16 g a.i. phosphonate at each of three injection periods (early flowering fruit/ fruit-set, mid-fruit-set, and immediately after harvest). In northern Queensland, durian shoot and root development appears to be active throughout the year despite the relatively cool conditions that occur during winter. Shoot-flushing activity often occurs in parts of the tree rather than uniformly over the canopy. Phosphonate was detected within two days of injection in all organs sampled and reached a peak between four and eight days after injection. The highest levels of phosphonate were recorded in leaves and flowers (mean value of 60 and 40 µg/g dry weight). Phosphonate levels either declined or increased with sampling date, depending on organ and injection time, but persisted in all tissues for at least 128 days. Phosphonate trunk injection trials were also carried out on local durian varieties in Vietnam. Under moderate disease pressure, annual injections of 16 g a.i. per tree gave superior control of canker compared with recommended sprays of metalaxyl or Aliette. Under high disease pressure, 48 g a.i., injected at 3 three-monthly intervals, gave the best disease control. Results presented in this paper demonstrate the efficacy of phosphonate in controlling phytophthora diseases in durian when applied as a trunk injection.

Introduction

In all regions where durian is grown, it is seriously threatened by diseases caused by *Phytophthora palmivora* Butl. This disease generally occurs on

mature fruit-producing trees. Symptoms include initial leaf-yellowing and leaf loss from the top of the canopy, with further loss of leaves occurring through the canopy at varying rates. New shoots may appear following initial severe defoliation, but further development and growth is unusual. Tree death generally occurs in 4–12 months from the initial onset of symptoms.

Attempts at controlling phytophthora diseases in durian have included repeated foliar sprays, or painting the cankered trunk with metalaxyl and phosphonate (salts or esters of phosphonic acid). These methods of application are expensive and the results highly variable under monsoonal conditions. Phosphonate is systemic and mobile in both xylem and phloem, and injection of the

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compound directly into the tree trunk has proved highly effective in controlling phytophthora diseases in a range of other tropical crops, including avocado, cocoa and coconut (Guest et al. 1995; Whiley et al. 1988).

Work in avocado has shown that, during periods of high vegetative flush and low root activity, phosphonate is carried up into the leaves rather than into the roots where it is required for the amelioration of *P. cinnamomi* (Whiley et al. 1995). Hence, the timing of injections in relation to tree phenology may be crucial to determining the distribution of the phosphonate within the durian tree and hence control of *P. palmivora*.

The experiments described in this chapter had three major objectives:

- to identify tree phenological activity under north Queensland environmental conditions with particular reference to the possibility of *P. palmivora* disease control using phosphonate injections;
- to monitor the distribution of phosphonate following trunk injection at three distinct phenological periods
- to identify the injection period which results in maximum uptake in all tree organs.

Finally, phosphonate was injected at a range of rates during different seasons into durian trees growing under a range of disease pressures in commercial orchards in Vietnam, to determine optimal application rates and timing.

Materials and Methods

Phenology monitoring

Three commercial farms and the Queensland Department of Primary Industries' (QDPI) South Johnstone research station, on the wet tropical coast of north Queensland, Australia, were selected as phenology recording sites. The sites were located within a region that extends from Bellenden Ker (16.5°S) in the north to an area south of Tully (18°S) a

distance of approximately 100 km. Five groups of mature trees (i.e. had flowered previously), each consisting of three trees of each of two cultivars (Luang and Montong), were chosen for monitoring depending on availability at each site. Tree phenology (shoot, root, flowering and fruiting activity) was monitored monthly for 30 months from January 2000 until June 2002. The monitoring sites and the sampling schedule are listed in Table 8.5.1.

Shoot activity was rated on a whole tree basis as a percentage of new, hardening or mature shoot (Figure 8.5.1). Flowering was rated on a scale of 0 to 3, with 0 = no flowers present, 1 = 1–20 flowers, 2 = 20–60 flowers and 3 = > 60 flowers present. Fruiting was also rated on a scale of 0–3 with 0 = no fruits, 1 = 1–10 fruits, 2 = 11–20 fruits and 3 = more than 20 fruits present. Harvest dates were recorded where applicable.



Figure 8.5.1 Durian flush standards, from left to right (new, maturing, mature).

Surface root activity was monitored through the use of 'root windows' (Figure 8.5.2a). The root windows consisted of a Perspex sheet (600 mm × 400 mm × 6 mm) installed on the SE side of each tree at a distance from the trunk equal to half the radius of the canopy. The perspex sheet was placed on a slope (5–35°) dependent on site topography, following soil removal and associated drainage. This process removed existing surface roots in the area. Before placing the

Table 8.5.1 Phenology monitoring sites, root window installation dates and sampling schedule

Farm	Variety	Install date	Sampling period during which monthly observations were made
CWTA	Luang	4/11/99	Jan 2000–June 2002
CWTA	Montong	14/12/99	Jan 2000–June 2002
Kuradui	Montong	23/11/99	Jan 2000–June 2002
Jensen	Montong	14/12/99	Jan 2000–June 2002
Zappala	Luang	11/11/99	Jan 2000–June 2002

perspex sheet, the face of the slope was covered in a fine layer of sterilised potting mix. The perspex sheet was held in place using steel pegs affixed to each corner. Each sheet was etched with corner markers to allow the placement of two A4 overhead projector acetate sheets. At each sampling, if unsuberised roots were present the overhead sheets were placed on the perspex sheet and root growth traced using a permanent marking pen. Between recording periods the perspex sheets were covered with newspaper, shade cloth and bags filled with hay to stop light penetration and insulate the roots from incident solar radiation. Root activity was assessed qualitatively. The qualitative method consisted of an activity rating of 0–2, where 0 = dormant roots, 1 = slight new growth and 2 = active new growth.



Figure 8.5.2 Root window installed under durian tree.

Phenology rating data were compiled and mean ratings were calculated per site and variety combination as well as across all varieties and sites. Variation is described by standard error. Climate data were collected at all four sites. Because of the similarity between climate data sets, only data collected at the South Johnstone research station are shown.

Phosphonate injection (Queensland)

An injection trial was carried out at the South Johnstone research station on the durian variety block. The block of 14-year-old trees consists of 14 cultivars, each cultivar replicated three times. The block is one of the few in north Queensland that has not been treated (injected or sprayed) with phosphonate. Although *P. palmivora* had been recorded on the trial site, trees showed no symptoms of the disease.

Injection times selected included:

- EFF – early flowering/fruit-set (7 October 2000), with the aim of getting phosphonate into

developing fruit, particularly fruit rind. Shoots and roots are also targeted

- MFS – mid-fruit-set (8 January 2001), with the aim of protecting all parts of the tree (shoot, root and possibly some protection to fruit)
- PH – immediately after harvest (26 March 2001), with the aim of avoiding direct flow of phosphonate to fruit, and distributing phosphonate to tops and possibly to roots during the last active phase of root development before root dormancy.

Three replicate trees were used per injection time, comprising three cultivars, Gumpun, Parung and Gob Yaow (all replicates of these varieties flowered and fruited during the 1998–99 season). Tree phenology was similar, and replicate trees of the same three varieties were used at each of the above injection times. The injection rate utilised was four 20 mL Chemjet® syringes of Foli-R-Phos® 200, which is equivalent to 16 g a.i. of phosphonate. Injections were administered in the early morning.

Sampling regime

All trees were sampled pre-injection on 21 September 2000. Post injection samples were obtained at 2, 4, 8, 16, 32, 64, 96, 128, 192, and 256 days. At each sampling date the following tree material was sampled:

- leaves (from lower, mid and upper canopy)
- composite bark and wood sample (lower, mid and upper trunk)
- flower/fruit samples (lower, mid and upper trunk) – where and when available
- root samples (0–15 cm depth) – eight per tree were subsampled and then bulked.

The leaf, bark/wood and flower/fruit samples were oven-dried at 40°C. Root samples were washed to remove all traces of soil before oven-drying at the above temperature. Following drying (2–3 days), samples were ground in a plant mill. A minimum of 5.0 g of dried ground material of each sample was packaged in labelled perspex containers and the collective samples were then air freighted to the University of Melbourne for analysis. Injection times and sampling dates are shown in Table 8.5.2.

Analysis

Phosphonate residues were measured by gas chromatography with a detection limit of 0.5 µg/g dry weight (dw).

Effect of phosphonate injection on disease in Vietnam

Phosphonate field trials were established on commercial orchards in the Mekong Delta and the Ba Ria-Vung Tau regions of Vietnam. In the Mekong

Delta, the efficacy of potassium phosphonate at different concentrations was compared with Aliette (aluminium tris-*O*-ethyl phosphonate) and Metalaxyl in 1–12 year-old durian cv. Kho qua xanh. Trunk injection was compared with foliar spray. Canker severity was measured on a scale of 0 (no canker) to 3 (trunk girdling more than 70%, or tree dead).

In Ba Ria–Vung Tau, the results of trunk injection with different concentrations of potassium phosphonate were compared with canker painting with Aliette in 4 or 7-year-old durian cv. Sua Hat Lep Ben Tre. Canker severity was measured on a scale of 0 (no canker) to 5 (canker more than 50 cm² or tree dead).

Results

Climate monitoring (Queensland)

Monthly maximum and minimum temperature, rainfall and evaporation totals and average shortwave solar radiation inputs are shown in Figure 8.5.3.

Over the 973-day period recorded there were 179 days where the maximum temperature was less than 25°C and 134 days where the minimum temperature was less than or equal to 15°C, with 26 days on which the recorded temperature was 10°C or less. The lowest temperature recorded was 7°C. The range in average temperature was from 14.5 to 31°C. These conditions are substantially cooler than durian trees experience in their native environment where the average temperature ranges from 24 to 30°C (Nanthachai 1994).

Total rainfall was 10,173 mm over 545 wet days, of which 53 days had rainfall equal to or above 50 mm. The corresponding total evaporation for the same period was 4889 mm. The driest months (monthly totals less than 50 mm) were July and September 2000 and May, July, August and December 2001 and

June 2002, when the respective rainfall recordings were 42, 24 and 36 and 37, 36, 43 and 16 mm. The wettest months (monthly totals greater than 500 mm) were December 1999, February, March, April and November 2000 and February 2001 when 505, 1121, 612, 948, 804 and 858 mm were recorded. These conditions, particularly during the first 24 months, are wetter than that experienced by the crop in its native environment where average rainfall ranges from 1600 to 4000 mm per year (Nanthachai 1994).

Energy inputs as measured by short wave solar radiation (SWSR) indicate that energy inputs varied across seasons. The average daily SWSR during the 973-day monitoring period was 18.6 MJ/m²/day, with a maximum daily influx of 29 MJ/m²/day and a minimum 6 MJ/m²/day. Monthly averages ranged from 12 to 24 MJ/m²/day. These variations are in part due to seasonal variation in day length and to a greater degree due to rainfall and associated cloud cover which occurs during the wet season. In general, clear days during the months September to October result in the highest incident SWSR.

In summary, the climate in the major north Queensland durian-growing areas is cooler and wetter than the climate in the natural growing environment of the fruit.

Phenology monitoring

Shoot activity was high throughout the monitoring period (Figure 8.5.4). The means, for all trees, show that during the 30-month monitoring period there were 10 months in which new shoot flush occurred on 40% or more shoots. Shoot growth occurred throughout the year, but the highest activity was generally recorded in the months leading up to summer (September–December). Flush activity during the winter months was generally below 40% and occurred in discrete patches within the canopy.

Table 8.5.2 Phosphonate injection and sampling schedule.

Days (pre/post injection)	1 st injection	2 nd injection	3 rd injection
Pre injection sample	21/9/00	21/9/00	21/9/00
Injection date	7/10/00	8/01/01	26/3/01
2	9/10/00	10/1/01	28/03/01
4	11/10/00	12/01/01	30/03/01
8	15/10/00	16/01/01	3/04/01
16	23/10/00	24/01/01	11/04/01
32	8/11/00	9/02/01	27/04/01
64	10/12/00	13/03/01	29/05/01
96	11/01/01	14/04/01	30/06/01
128	12/02/01	16/05/01	1/08/01
192	17/04/01	19/07/01	4/10/01
256	20/06/01	21/09/01	7/12/01

Trees at individual sites exhibited similar flushing patterns.

Flower and fruiting activity varied between seasons (Figure 8.5.5). In the 2000 season, the spread of flowering was relatively short and intense, with a peak from September to October.

In the 2001 season, flowering at three of the five sites occurred over a longer period (May 2001–January 2002), continuing until May 2002 at one of the sites. The longer flowering period in 2001 may have been

due to the drier conditions (Figure 8.5.3), which occurred from July 2001 to December 2001. fruit-set and growth closely followed flowering, with fruit harvest occurring from January 2001 to March 2001 in the 2000–2001 season and from January 2002 to May 2002 in the 2001–2002 season. fruit-set at one site (SJ-Monthong) was particularly poor in the 2001–2002 season.

In trees monitored in north Queensland root activity varied greatly between sites (Figure 8.5.6). Peaks in activity tended to occur during summer, but some

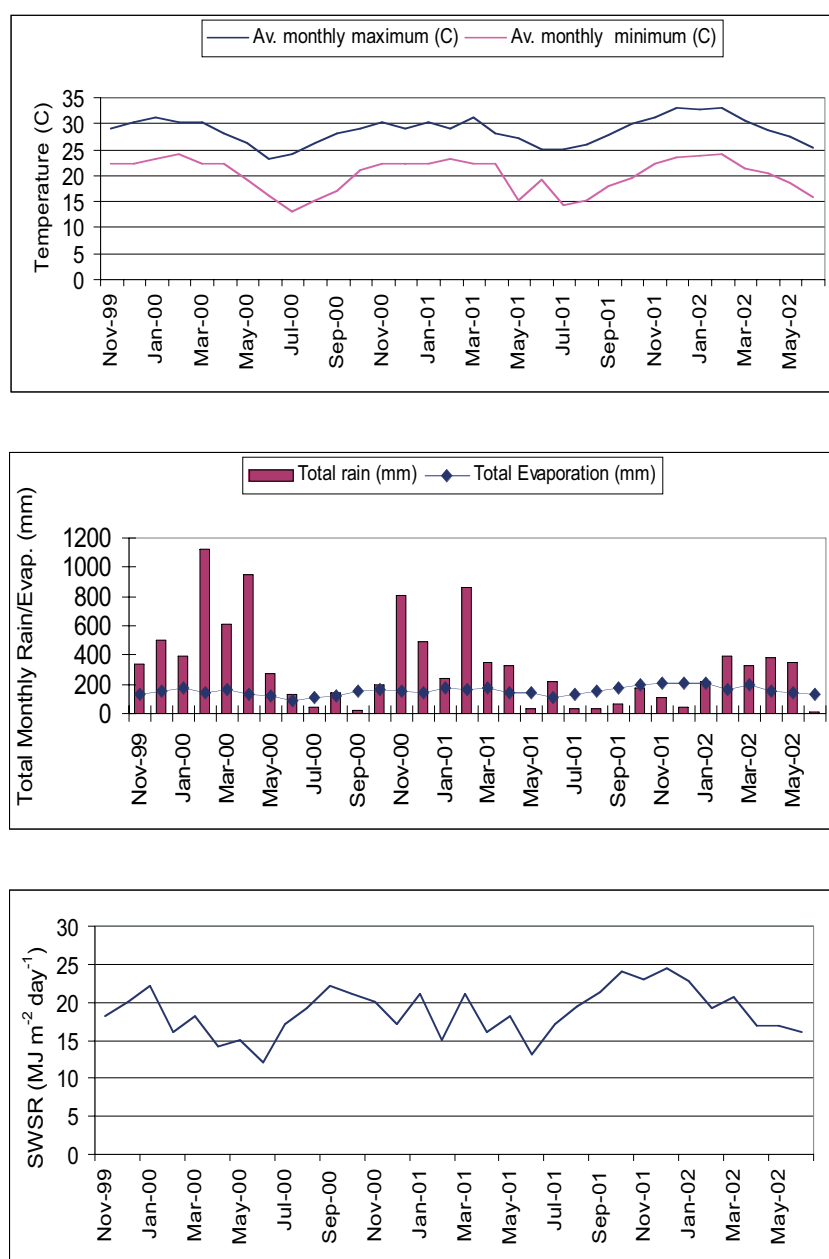


Figure 8.5.3 Mean monthly maximum and minimum temperature (°C), total monthly rain (mm) and evaporation (mm) and mean monthly shortwave solar radiation (MJ m²/day) recorded at South Johnstone, northern Queensland, during the phenology monitoring period.

activity was noted throughout the year. The one period noted for a lack in activity in four of the five sites (May 2000–August 2000) corresponded with consistent cool conditions.

Translocation of phosphonate

Phosphonate concentration data from three injection periods have been analysed (early flowering/fruit-set, mid-fruit-set and postharvest). Phosphonate was not detected in any of the pre-injection samples of tissue, but was detected in all tissues within 2 days of injection (Figure 8.5.7). The concentration of phosphonate in organs was highest between 4 and 16 days after injection and generally fell below 10 $\mu\text{g/g}$ dry weight 65 days after injection.

Phosphonate concentrations increased in bark/wood samples from 96 to 256 days after the early flowering/fruit-set injection, whereas they remained relatively high following the mid-fruit-set injection.

The highest concentrations of phosphonate were recorded in leaves and bark wood (mean values of 134 and 105 $\mu\text{g/g}$, respectively) within 8 days of injection at the postharvest injection. However, there were little differences in the concentration between organs as the variability within the leaf samples was very high (Figure 8.5.7), with no detectable residue in some samples and more than 200 $\mu\text{g/g}$ dw in others. In trees injected during mid-fruit-set, mean phosphonate concentrations never exceeded 30 $\mu\text{g/g}$ dw. Variability within organs was lower, but a peak in phosphonate concentrations (8 days after injection) was discernible only in the leaf samples. Mean phosphonate concentration in roots was generally low (≤ 10 $\mu\text{g/g}$ dw), but in the postharvest injection treatment, concentrations in roots ranged from 21 to 44 $\mu\text{g/g}$ dw from 4 to 32 days after injection.

Effect of phosphonate injection on disease in Vietnam

At sites of moderate disease pressure in the Mekong Delta Region, canker healing was observed within 4 months of injecting trees with 16 g a.i. phosphonate (applied as a single injection in April). Cankers continued to heal over the following 8 months until they had a canker rating of less than 1. Canker healing was achieved in other sites in the Mekong Delta Region with 32 g a.i. phosphonate (applied in two injections of 16 g a.i. with a 5-month interval). Under heavy disease pressure, 48 g a.i. per tree, along with pruning, improved drainage and orchard hygiene, gave the best disease control.

Phosphonate (0.2 or 0.4 g a.i./L), Aliette (1.6 g a.i./L) or metalaxyl (1.6 g a.i./L) significantly reduced

preharvest fruit rot when applied as foliar/fruit sprays 1 month before harvest in the Mekong Delta. However, sprays of phosphonate applied at 0.4 g a.i./L or Aliette at 1.6 g a.i./L gave significantly superior control (Table 8.5.3).

Table 8.5.3 Average fruit yield and preharvest rot from 6-year-old durian trees in Vung Tai–Ba Ria, Vietnam, one year after treatment; $n = 20$. Values within columns are shown to be significantly different by ANOVA, $P = 0.05$

Treatment	Average yield (kg/tree)	Percentage fruit rot
Water injection	11.4a	43.7a
Phosphonate injection 12 g	25.5b	10.5c
Phosphonate injection 18 g	26.7b	13.5b
Phosphonate injection 24 g	27.3b	12.0bc

In Vung Tau–Ba Ria, canker healing was achieved in 4-year-old trees with either one or two applications of 8 g a.i. phosphonate per tree per year, while canker painting did not significantly reduce cankers (Figure 8.5.8). In 6-year-old trees 3 injections at 3-month intervals with 8 g a.i. or 2 injections (6-month interval) of 8 g a.i., gave superior control to a single injection of 12 g a.i. All of the above treatments resulted in a significantly higher yield of healthy fruit. Excellent control was also achieved in 7-year-old trees with 3 injections totalling 16, 24, 32 g a.i. of phosphonate per tree per year, compared with Aliette 80 WP 1% paint, with 32 g a.i. treatment the most effective.

Discussion

Flushing, flowering and fruiting patterns of durian recorded in north Queensland are similar to patterns observed in Malaysia and Thailand. Higher rates of leaf flushing occur during the wet season, while flowering normally occurs during or near the end of the dry spring months, and fruit development and harvest during the wet summer months (Subhadrabandhu and Ketsa 2001). Thai researchers report that the ideal temperature range for durian production is from 24°C to 30°C (Nanthachai 1994, Subhadrabandhu and Ketsa 2001). This study has revealed that active vegetative growth can occur under relatively cool conditions (three months where mean temperatures range from 18.5°C to 20°C and seven months where mean temperatures were >20°C and less than 24°C) as experienced in north Queensland. Surprisingly, root growth also continues during this period. In north Queensland, observations on durian root distribution agree with data presented by Masri (1991) showing that the durian root length density decreased horizontally

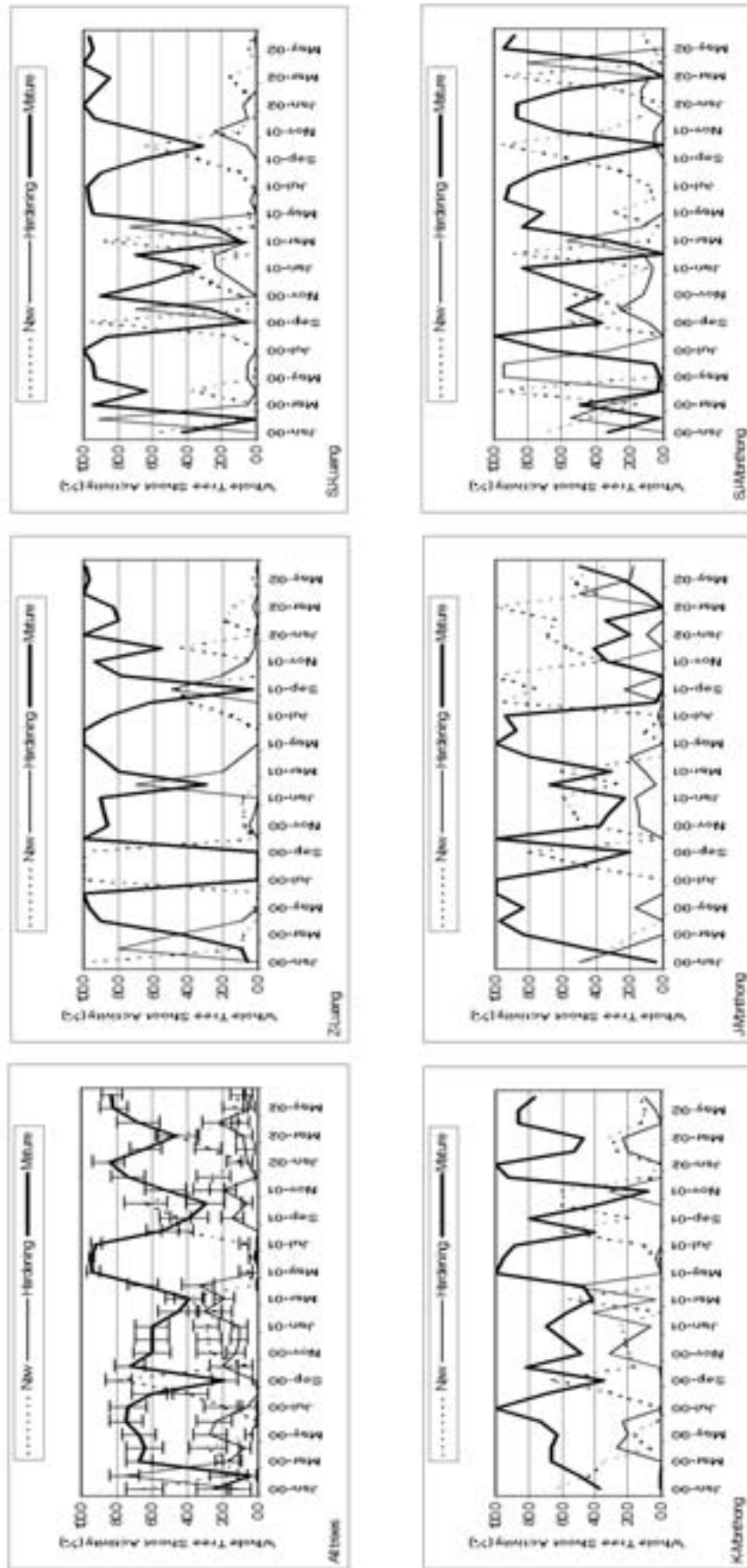


Figure 8.5.4 Whole tree shoot phenology (%) for individual farm sites (Z-Luang, S-Luang, K-Monhthong, J-Monhthong, S-Monhthong) and all trees plus standard error bars.

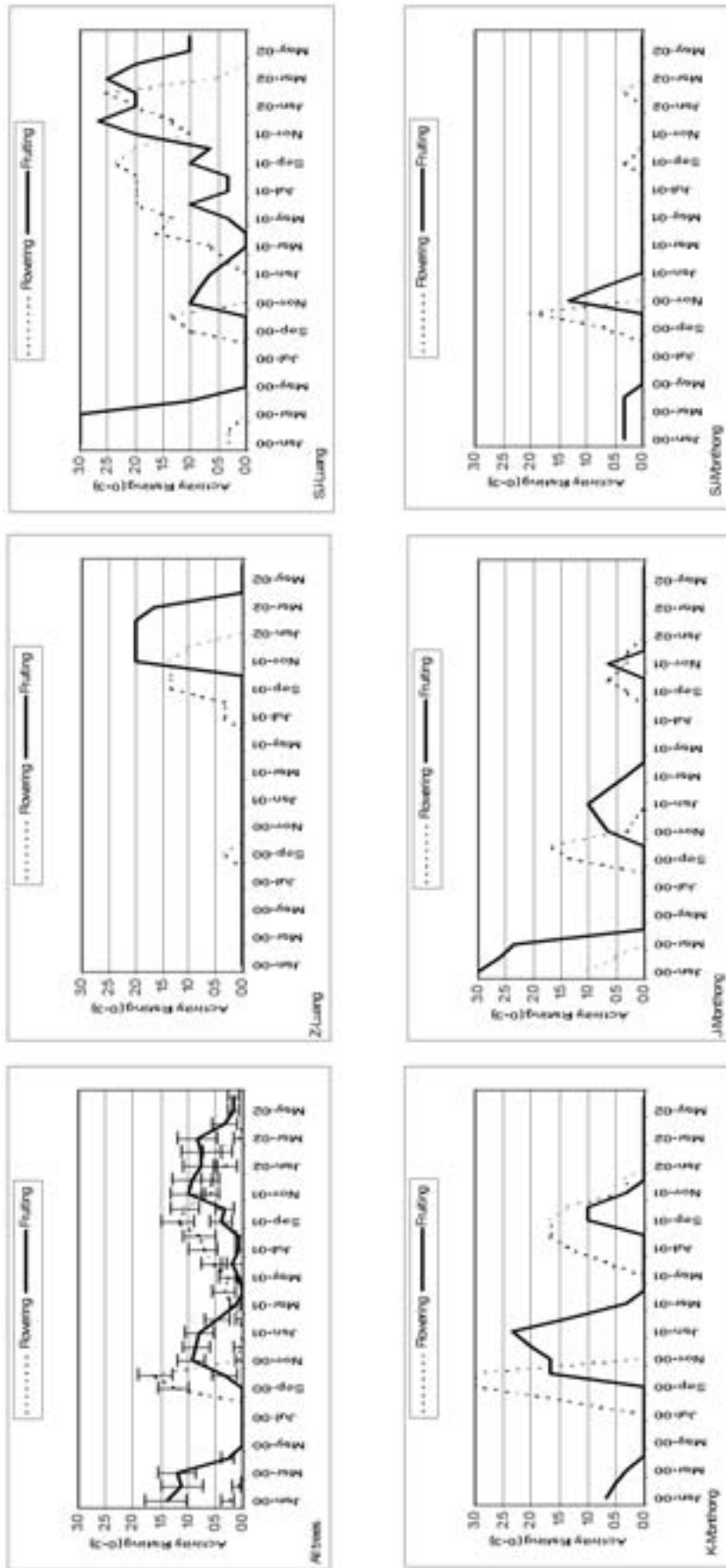


Figure 8.5.5 Flowering and fruit-set activity rating for individual farm sites (Z-Luang, SJ-Luang, K-Monthong, J-Monthong, SJ-Monthong) and all trees plus SE bars.

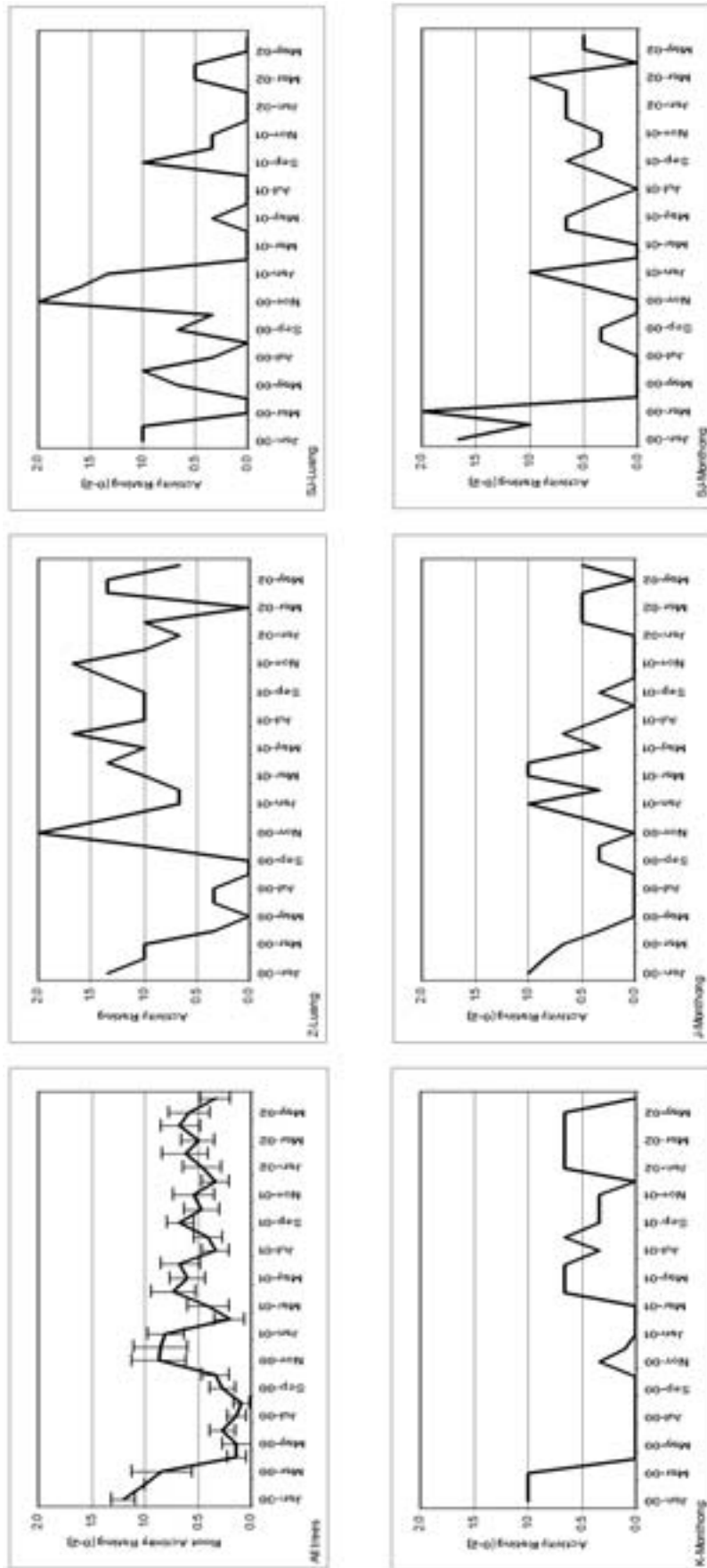


Figure 8.5.6 Root growth activity rating for individual farm sites (Z-Luang, S1-Luang, K-Monthon, J-Monthon, S2-Luang) and all trees plus SE bars.

from the crown and vertically with soil depth but no data have been found that document root flushing activity.

The continuous growth of shoots and roots observed in durian differs from avocado where shoot and root activities have two distinct growth stages with the root growth following shoot growth (Whiley et al. 1988). Our data suggest that new shoot and root activity in durian occur simultaneously or are only slightly offset.

Phosphonate concentrations recorded in durian in this trial are lower than those observed in similar studies conducted in avocado (Whiley et al. 1995). In avocado, concentrations of phosphonate were as high as 80 $\mu\text{g/g}$ fresh weight (fw) and 25 $\mu\text{g/g}$ fw in

shoots and roots, respectively. Equivalent fresh weight maximum concentrations in durian were 24 $\mu\text{g/g}$ and 2.3 $\mu\text{g/g}$ for shoots and roots.

The phenological patterns observed suggest that shoot and root growth occurs throughout the year, albeit at higher levels during the summer months. This suggests that translocation of phosphonate to all developing meristems is possible regardless of the time of injection, unlike the situation in avocado where maximal levels in roots could be achieved only if injections followed the maturity of the spring shoot growth (Whiley et al. 1995). Surprisingly, phosphonate levels in durian generally remained low in roots (less than 10 $\mu\text{g/g}$ dw). This suggests that either the root sink strength is low or the

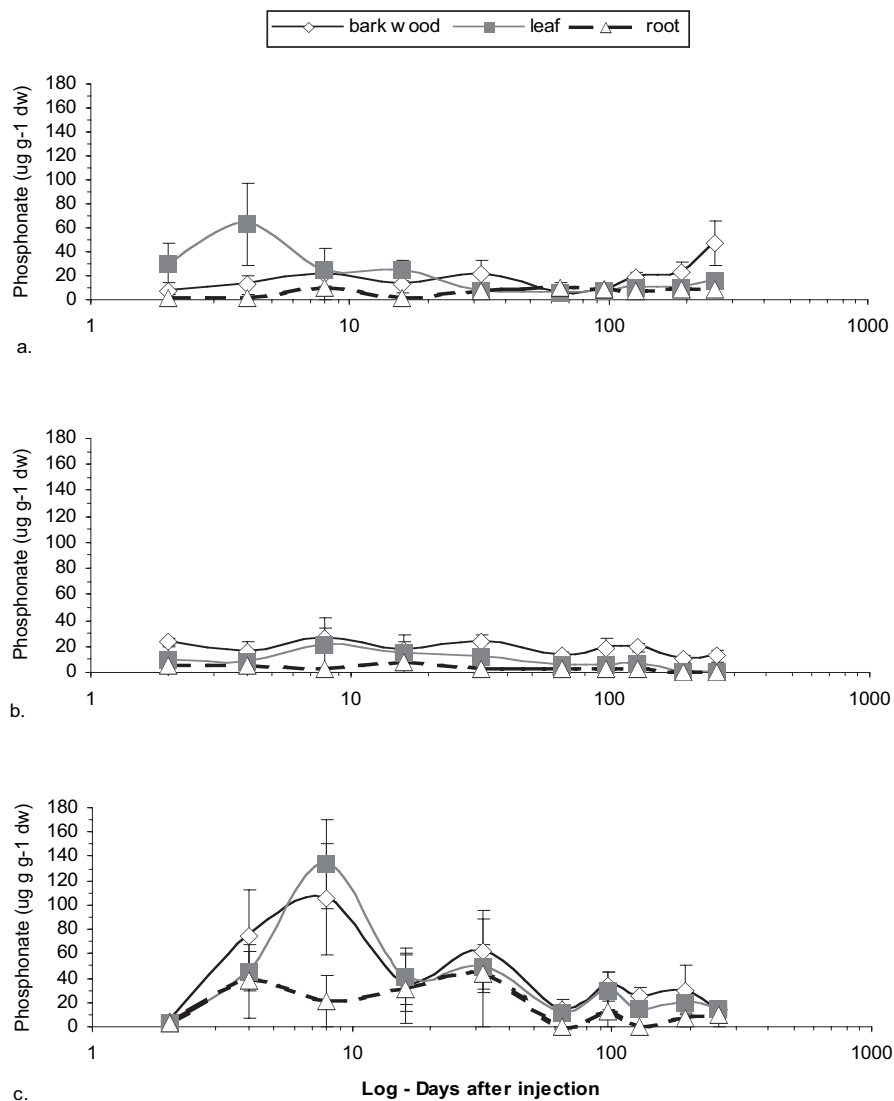


Figure 8.5.7 Phosphonate concentrations in durian tissue following injection after a) early flower and fruit-set, b) mid fruit-set and c) immediately post harvest with 16 g a.i. phosphonate.

concentration of phosphonate injected is inadequate to supply all organs simultaneously. Concurrent work in Vietnam has shown that the concentrations used in this experiment are sufficient to halt the development of stem canker. In this study, the phosphate concentrations were highest in the bark/wood samples following the mid-fruit-set injection. There were, however, no symptoms of bark canker observed in the trees before or during the sample period in north Queensland.

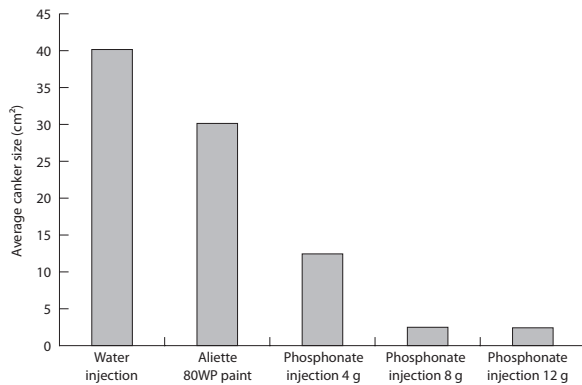


Figure 8.5.8 Severity of canker symptoms on 4-year-old trees in Vung Tau-Ba Ria, Vietnam, 1 year after treatment; $n = 20$.

Phosphonate trunk injections effectively and consistently control durian trunk canker in trials conducted under high disease pressure in Vietnam, and lead to increased healthy fruit yield, as they do in cocoa and coconut. When they are used in conjunction with improved orchard hygiene, canopy management, drainage and preharvest foliar sprays of either phosphonate or Aliette, one could expect greater control of fruit rot. The optimal rate of application depends on disease severity and disease pressure. Trials conducted over 5 years on cocoa in Papua New Guinea using trunk injections of potassium phosphonate increased healthy pod yield and decreased the incidence of *Phytophthora* pod rot when compared with untreated trees or trees sprayed with recommended doses of Ridomil 250 EC or trunk injected with Aliette CA (Guest et al. 1994). A single annual injection of 15 g a.i. per tree controlled *Phytophthora* disease on mature cocoa trees, with the optimal dose depending on tree size, initial disease severity and disease pressure.

In conclusion, durian shoot and root growth remains relatively active throughout the year. This may be beneficial in terms of *Phytophthora* disease control via the mechanism of phosphonate trunk injection because sink strength remains active in all growing organs throughout the year. However, because of the absence of disease in north Queensland where we monitored the effect of phenology on tissue concentrations of phosphonate, we can only infer that these concentrations are adequate to explain the excellent level of disease control achieved in the trials conducted in Vietnam.

Acknowledgments

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8.6 Control of Postharvest Diseases in Durian

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Abstract

Disease incidence and disease severity associated with *Phytophthora palmivora* and other fungi was greater in fruit that had contact with soil during harvest, and when postharvest storage conditions were 15°C and 90% relative humidity. Other fungi isolated from symptomatic fruit stored under ambient conditions included *Fusarium* sp., *Mucor* sp. and *Botryodiplodia* sp. Preharvest sprays of durian fruit with 2 g/L fosetyl-al significantly reduced postharvest disease incidence and symptom severity compared with water-treated controls. A combination of preharvest spray and postharvest fruit dip of 1 g/L a.i. fosetyl-Al gave the best disease control. A postharvest dip of fruit in 1 g/L a.i. fosetyl-Al did not reduce postharvest rot.

Introduction

Much of the literature on phytophthora disease control in durian concentrates on the treatment of patch or trunk canker. However, the development of distant and international markets has also made consideration of postharvest fruit health a priority. While *Phytophthora palmivora* is the most serious pre- and postharvest pathogen of durian, *Sclerotium rolfsii*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides* and *Fusarium solani* also reduce the shelf life and value of the fruit (Lim 1990; Nanthachai 1994).

Harvesting indices developed for Thai varieties enable early harvesting, which gives time for transport of the fruit to distant markets before ripening. Harvesting indices are not relevant to the Vietnamese durian industry, as there is currently a high level of variability in the planting material.

The most recent survey of durian diseases in Vietnam puts postharvest losses of durian due to

phytophthora diseases at up to 15%, but they may be as high as 30%, and locally can be devastating when whole consignments are lost through transit rot (Lim 1990; Lee 1994). Vietnam’s durian industry is small and currently caters mainly to the local market and durian-growing areas in the south-east of Vietnam are close to major population centres (Dr Nguyen Minh Chau, Director, Southern Fruit Research Institute (SOFRI), pers. comm.). Both farmers and the government aim to develop the export potential of this high-value crop to meet increasing international demand for the fruit. Consequently, the area under durian cultivation is expanding rapidly in Vietnam, in some cases into marginal lands, and recommendations for phytophthora disease control are urgently needed. The research presented in this chapter examines methods of postharvest disease control using pre- and postharvest treatments of phosphonate, which have proven highly effective in controlling phytophthora trunk canker in durian (Chapter 8.5), and associated diseases in other crops (Guest et al. 1995; Konam 1999)

Materials and Methods

Effect of harvest method on postharvest disease development

Two harvesting methods were compared on durian cv. Kho Qua Xanh at two times during the fruiting

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season of 2000; early in the season (February) and at the peak of the season (May–June). The two harvesting methods compared were:

- fruit fall, simulated by cutting the fruit from the branch and dropping it to the ground from a height of 3 m
- cut and collect, where ripe fruit was cut from the branch and carefully packed into boxes with no soil contact.

Harvested fruit was transported to the laboratory, where it was stored for 3 weeks either under ambient conditions ($n = 5$), or in controlled-environment chambers at 15°C and 90% relative humidity (RH) ($n = 10$).

To determine disease incidence, symptomatic tissue was excised, surface sterilised and plated onto potato dextrose agar, and the causal agent identified through morphological characteristics. Symptom severity was rated on a scale of 0–4: 0 = no symptoms, 1 = lesions covering 1–5% of the fruit, 2 = lesions covering 6–10% of the fruit, 3 = lesions covering 11–20% of the fruit, and 4 = lesions covering more than 20% of the fruit. The severity for each treatment was calculated using the following formula:

$$\text{Severity} = \sum(\text{severity rating} \times \text{rating frequency})/n$$

Effect of preharvest fungicide spray on postharvest disease development

These experiments were carried out between March and June 2000 at Cai Lay District, Tien Giang Province in Vietnam, on durian cv. Kho Qua Xanh. The three preharvest fruit spray treatments were:

- 1) 1 g/L a.i. fosetyl-al (Aliette 80 WP, Bayer CropScience)
- 2) 2 g/L a.i. fosetyl-al
- 3) water (control).

Treatments were applied directly to the fruit 30 days after fruit set, and again after a 30-day interval. There were 5 trees per treatment and 10 fruit harvested from each tree, followed by transport to SOFRI and storage in a controlled-environment chamber at 15°C and 90% RH for 3 weeks. Disease incidence and symptom severity were calculated as described above.

Effect of postharvest fungicide dip on postharvest disease development

Experiments were conducted between May and July 2000, on mature fruits of durian cv. Kho Qua Xanh harvested from durian orchards in Tien Giang Province and transported to SOFRI. The five postharvest fruit dip treatments were:

- 1) 1 g/L a.i. fosetyl-al
- 2) 2 g/L a.i. fosetyl-al
- 3) 3 g/L a.i. fosetyl-al
- 4) 4 g/L a.i. fosetyl-al
- 5) water (control).

There were 5 fruit per treatment. Fruit was immersed in the treatment solution for 5 minutes, dried at ambient temperature and stored for 3 weeks in a controlled-environment chamber at 15°C and 90% RH, and a further 2 days under ambient conditions. Disease incidence and symptom severity were calculated as described above.

Effect of combining pre- and postharvest fungicide treatments on postharvest disease development

This experiment was also carried out between March and June 2000 at Cai Lay District, Tien Giang Province, on durian cv. Kho Qua Xanh. Six treatments were applied as described above, in the following combinations:

- 1) preharvest spray with 2 g/L a.i. fosetyl-al
- 2) preharvest spray with water
- 3) postharvest dip in 1 g/L a.i. fosetyl-al
- 4) postharvest dip in water
- 5) preharvest spray with 2 g/L a.i. fosetyl-al and postharvest dip in 1 g/L a.i. fosetyl-al
- 6) preharvest spray with water and postharvest dip in water.

Treated fruit were stored in a controlled-environment chamber at 15°C and 90% RH for 15 days, and a further 4 days under ambient conditions. Disease incidence and symptom severity were calculated as described above.

Data analysis

Data were analysed by analysis of variance (ANOVA) and least significant differences (LSD) computed at 5% and 1% levels of significance, in order to test differences between means. Results are presented as the LSD between means that would be significant under the conditions of the test.

Results

Effect of harvest method on postharvest disease development

When fruit were harvested to avoid contact with orchard soil, no disease symptoms developed within 21 days of fruit being stored at ambient temperature. When soil contact was allowed during harvest, there was a greater disease incidence in fruit harvested at peak season than fruit harvested early in the season. *P. palmivora* was not isolated from any fruit stored at

ambient conditions, regardless of harvest date or method of harvest (Table 8.6.1).

Disease incidence and disease severity associated with *P. palmivora* and other fungi was greater in fruit that had contact with soil during harvest, when postharvest storage conditions were 15°C and 90% RH (Table 8.6.2). Other fungi isolated from fruit with disease symptoms stored under ambient conditions included *Fusarium* sp., *Mucor* sp. and *Botryodiplodia* sp.

Effect of preharvest fungicide spray on postharvest disease development

Preharvest sprays of durian fruit with fosetyl-al significantly reduced postharvest disease incidence and symptom severity compared with water-treated controls. There was no significant difference in disease incidence or symptom severity between the two rates of fosetyl-al used (Table 8.6.3), although the cause of disease symptoms was not identified.

Table 8.6.1 The percentage of durian cv. Kho Qua Xanh fruit exhibiting disease symptoms from infections by *Phytophthora palmivora* or other fungi after harvest in February 2000 (early season) or May–June 2000 (peak season) by one of two harvesting methods: (a) fruit fall – where fruit came into contact with orchard soil and (b) cut and collect – where no soil contact was allowed, followed by storage under ambient conditions. $n = 5$.

Harvest method	Number of diseased fruit after 21 days			
	Early season		Peak season	
	<i>Phytophthora</i>	Other fungi	<i>Phytophthora</i>	Other fungi
Fruit fall	0	1	0	3
Cut and collect	0	0	0	0

Table 8.6.2 The percentage of Kho Qua Xanh variety of durian fruit exhibiting disease symptoms, and mean symptom severity resulting from infections by *Phytophthora palmivora* or other fungi after peak season harvest (May–June 2000) by one of two harvesting methods: (a) fruit fall – where fruit came into contact with orchard soil, and (b) cut and collect – where no soil contact occurred, followed by storage at 15°C and 90% RH for 3 weeks. $n = 10$.

Harvest method	Disease incidence (%)		Severity ¹	
	<i>Phytophthora</i>	Other	<i>Phytophthora</i>	Other
Fruit fall	16.7a	27.1a	1.96A	1.72A
Cut and collect	4.0a	15.0b	0.82B	0.72B
LSD _{0.05}	16.0	9.7	–	–
LSD _{0.01}	–	12.7	0.63	0.92

¹ Severity rated on scale 0–4 according to percentage of fruit surface with lesions: 0 = no lesions, 1 = 1–5%, 2 = 6–10%, 3 = 11–20% and 4 = >20%.

Means followed by the same lower case letter are not significantly different according to LSD (0.05).

Means followed by the same upper case letter are not significantly different according to LSD (0.01).

Table 8.6.3 The percentage of durian cv. Kho Qua Xanh fruit exhibiting disease symptoms, and mean symptom severity after two preharvest fruit sprays (30-day interval) with fosetyl-al (Aliette 80 WP) followed by manual harvest and storage for 3 weeks at 15°C and 90% RH. $n = 50$.

Treatment	Disease incidence (%)	Severity ¹
Water (control)	40.0A	1.20a
fosetyl-Al g/L	12.0B	0.32b
fosetyl-Al 2 g/L	8.0B	0.12b
LSD _{0.05}	–	0.68
LSD _{0.01}	23.2	–

¹ Severity rated on scale 0–4 according to percentage of fruit surface with lesions: 0 = no lesions, 1 = 1–5%, 2 = 6–10%, 3 = 11–20% and 4 = >20%.

Means followed by the same lower case letter are not significantly different according to LSD (0.05).

Means followed by the same upper case letter are not significantly different according to LSD (0.01).

Effect of postharvest fungicide dip on postharvest disease development

Although postharvest dipping of durian fruit into fosetyl-al solutions of up to 4 g/L a.i. significantly reduced disease symptom severity compared with the water control, it did not significantly reduce the incidence of disease. There was no significant difference in symptom severity between the different concentrations of fosetyl-al tested (Table 8.6.4). Again, the cause of disease symptoms was not identified.

Effect of combining pre- and postharvest fungicide treatments on postharvest disease development

Preharvest spray with 2 g/L a.i. fosetyl-Al reduced the postharvest disease incidence and symptom severity in durian, while postharvest dip of fruit in 1 g/L a.i. fosetyl-Al did not. A combination of these pre- and postharvest treatments gave the best disease control (Table 8.6.5), although the cause of fruit rot was not identified.

Discussion

The results of this study demonstrate the importance of minimising contact between fruit and soil during harvesting, not only in controlling postharvest phytophthora diseases but also those caused by other fungi. An added advantage of harvesting the fruit from the tree is the prevention of impact damage as the ripe fruit hits the ground on abscission. Durian that is allowed to separate naturally is believed to have a better flavour than harvested fruit, so farmers in Malaysia, Indonesia and the Philippines tie the fruit to the branches so that it can separate without the associated problems of natural drop (Figure 8.1.7; Nanthachai 1994). In recent years, farmers in Vietnam have also adopted this practice. In Thailand, harvesting of mature but unripe fruit is commonly undertaken by a skilled team; one person climbs into the tree and cuts the stalk, allowing the fruit to drop to a second person on the ground, who catches it in a jute sack (Figure 8.1.7).

Table 8.6.4 The percentage of durian cv. Kho Qua Xanh fruit exhibiting disease symptoms, and mean symptom severity after postharvest dip for 5 minutes in 1, 2, 3, or 4 g/L a.i. fosetyl-Al (Aliette 80 WP, or water (control), followed by storage for 3 weeks at 15°C and 90% RH, and a further 2 days under ambient conditions. $n = 5$.

Treatment	Disease incidence (%)	Severity ¹
Water (control)	15a	0.85a
fosetyl-Al 1 g/L	15a	0.40b
fosetyl-Al 2 g/L	10a	0.25c
fosetyl-Al 3 g/L	5a	0.15c
fosetyl-Al 4 g/L	10a	0.25c
LSD _{0.05}	-	0.15

¹ Severity rated on scale 0–4 according to percentage of fruit surface with lesions: 0 = no lesions, 1 = 1–5%, 2 = 6–10%, 3 = 11–20% and 4 = >20%.

Means followed by the same lower case letter are not significantly different according to LSD (0.05).

Table 8.6.5 The percentage of durian cv. Kho Qua Xanh fruit exhibiting disease symptoms, and mean symptom severity after preharvest spray with 2 g/L a.i. fosetyl-Al (Aliette 80 WP), postharvest dip in 1 g/L a.i. fosetyl-Al, or a combination of the two. Control fruits were similarly treated with water. After treatment fruit was stored for 15 days at 15°C and 90% RH, and a further 4 days under ambient conditions. $n = 5$.

Preharvest spray	Postharvest dip	Disease incidence (%)	Severity ¹
Water	Water	84.4d	1.81a
Water	-	50.0c	0.97b
-	Water	53.1ac	1.09b
2 g/L fosetyl-Al	-	28.1a	0.41c
-	1 g/L fosetyl-Al	37.5a	0.59c
2 g/L fosetyl-Al	1 g/L fosetyl-Al	12.5b	0.25c
LSD _{0.01}		27.56	0.39
LSD _{0.05}		19.93	0.55

¹ Severity rated on scale 0–4 according to percentage of fruit surface with lesions: 0 = no lesions, 1 = 1–5%, 2 = 6–10%, 3 = 11–20% and 4 = >20%.

Means followed by the same lower case letter are not significantly different according to LSD (0.05).

The current study shows that disease incidence was greater at peak season (May–June) than at the start of the season (February). This is a not unexpected finding, as February in southern Vietnam is hot and dry and fruit is in the early stages of development, while May–June is during the monsoon with high levels of humidity coupled with an ample energy source for pathogens in the ripening fruit.

Phytophthora symptoms did not develop on fruit that was stored under ambient conditions, regardless of time or method of harvest, but did develop when fruit was stored at 15°C and 90% RH for 3 weeks. Prolonged periods of high humidity seem to be the key here, as 98% RH had to be maintained for at least 72 h for disease development to occur in non-wounded, artificially inoculated durian fruit (Chapter 3.2).

In the Ba Ria–Vung Tau region of Vietnam, Mr Mai Van Tri and colleagues clearly demonstrated that trunk injection with phosphonate not only ameliorates phytophthora trunk canker but also reduces the incidence of preharvest diseases, with a consequent increase in the yield of healthy fruit (Chapter 8.5). Phosphonate also reduces the incidence and severity of postharvest diseases in durian when applied as a preharvest spray during the fruit development period, with a follow-up postharvest dip. A preharvest spray with phosphonate without any postharvest treatment will afford some protection. Although a postharvest dip on its own may reduce symptom severity, it is not effective in reducing the incidence of disease. Nanthachai (1994) cites unpublished work from Thailand that confirms the effectiveness of combining pre- and postharvest treatments of phosphonate for the control of postharvest diseases.

Nanthachai (1994) expressed some concern about the use of phosphonate in fruit disease control due to limited knowledge about the effect of residues. However, the Australian Pesticides and Veterinary Medicines Authority (formerly the National Registration Authority), which is the national registration authority for agricultural and veterinary chemicals, recently declared that residue data are not required for the registration of phosphonate in Australia, due to the biologically benign nature of the formulas to non-target organisms (Guest and Grant 1991; NRA 2001). Taste-tests revealed that injected phosphonate had no adverse effects on fruit palatability.

Recommendations for the control of postharvest diseases in durian have been formulated through this study. The following control measures should be included into a broader, integrated regime of management for the crop:

- minimisation of inoculum levels in the orchard through the regular removal and destruction of diseased branches and fruit
- minimisation of inoculum levels in the orchard by control of patch canker with phosphonate trunk injections according to recommendations in Chapter 8.5
- control of insects that may carry inoculum into the tree canopy
- reduction humidity in the orchard through pruning to improve airflow
- phosphonate treatment: fruit spray with 2 g/L a.i. fosetyl-Al during fruit development and again 30 days later, followed by a postharvest dip in 1 g/L a.i. solution of fosetyl-Al
- manual harvesting of fruit that prevents fruit coming in contact with the soil
- careful postharvest handling of fruit to prevent injury and development of pathogen infection courts.

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8.7 Integrated Management of Phytophthora Diseases of Durian: Recommendations and Benefit–Cost Analysis

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Abstract

Durian is a favourite fruit throughout Southeast Asia. Increasing areas have been planted to durian orchards in recent years, especially in the Mekong Delta and southeastern provinces of Vietnam, in marginal areas of Thailand and in northern Australia. Durian growers face significant losses due to phytophthora diseases, and there is an urgent need for recommendations to control these diseases. Integrated disease management recommendations, based on an understanding of the biology of the pathogen, optimal growing conditions and soil health, promise sustainable durian production with minimal environmental impact. We have developed integrated orchard management recommendations based on an appreciation of the natural rainforest conditions in which durians co-evolved with the pathogen.

Introduction

Phytophthora is a serious pathogen of durian that has the ability to attack the plant at various stages of its life cycle. Roots, stems and leaves of seedlings, young trees and mature trees are affected, as well as flowers and fruit. *Phytophthora palmivora* is a pathogen on a wide range of host plants grown throughout Southeast Asia. Major epidemics occurred in 1994 in Thailand, and in 2001 in Vietnam. Hence, it is easy to understand that to

control *P. palmivora* in durian, we need an integrated approach that takes the disease cycle, host range and cultivation practices for durians into account.

Integrated disease management (IDM) is the long-term reduction of disease losses to economically acceptable levels through a holistic approach that combines the use of resistant varieties, cultural control methods, biological control methods, and the judicious application of appropriate chemicals. The principle of integrated management of phytophthora diseases in durian has been promoted since the early 1990s (Lim 1990; Bong 1993; Lee 1994), but detailed recommendations appropriate for all regions have been lacking, and subsequent implementation patchy. A systematic approach to developing recommendations was undertaken as part of an ACIAR-funded project, 'Management of *Phytophthora* diseases in durian' (Project no. PHT/1995/134), which commenced in 1998. As part of the project, practical disease-control options were investigated, regionally optimised and disseminated to durian farmers in Thailand, Vietnam and Australia. The project culminated in a

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workshop in Chiang Mai, Thailand in November 2002, discussions at which formed the nucleus for the production of this monograph.

The recent and rapid expansion of the durian industries in Thailand and Vietnam has seen the establishment of orchards on increasingly marginal sites, including rice paddy in Vietnam (Figure 6.7.9), where phytophthora diseases can be exacerbated. Sources of disease resistance in durian and the development of tolerant rootstocks have yet to be identified, although the screening techniques described in Chapter 8.3 should facilitate the search. Nursery standards have to be improved to ensure that infected planting material is not released to growers (Chapter 8.4).

In the past, gaps in our understanding of the epidemiology of *P. palmivora* in durian have hampered effective management, and have resulted in the application of inappropriate and ineffective management practices. Although effective against phytophthora diseases of avocado and cocoa, the lack of specific recommendations for the rate and timing of phosphonate trunk-injection of durian have so far limited efficient application and effective disease control using this technique.

Integrated disease management of durians aims to minimise infection at various points in the disease cycle. Initially, this includes using clean, disease-free planting material and properly prepared planting sites. After establishment of an orchard, management priorities include improving and maintaining soil health through the use of organic matter and green manure, manipulation of soil moisture and drainage, and correct nutrient management. Care must be taken to prevent the spread of soil-borne inoculum into the canopy.

Disease development can also be slowed down through the removal of infected fruit from the canopy and by general orchard hygiene. If stem cankers are active, they may be treated with phosphonate injections to cure them. Details of the various components of the IDM practice developed are given below.

Planting and Pruning

Farmers should select disease-free planting stock from a reputable nursery. Grafted seedlings can be useful if disease-resistant rootstocks are available, or if the farmer wants to multiply an elite, selected scion cultivar. Avoid planting directly on old rubber, cocoa or pawpaw land, as these plants are susceptible hosts for *Phytophthora palmivora*, and high levels of soil inoculum may have built up. If this

is not possible, grow a legume groundcover for at least one year before transplanting durian, slash the green vegetation and use as a green manure to build-up soil organic matter and microbial activity.

If the green manure is fermented or composted it may also suppress existing *Phytophthora* infestations of the planting hole. One technique is to excavate a 2 m diameter by 50 cm deep planting hole, fill it with green manure, add fresh chicken manure and a microbial starter culture such as EM (Effective Microorganisms, <<http://www.emtrading.com/index.html>>), trample to remove air, and cover with compacted soil. Leave the material to ferment for 8–10 weeks, before forming into a mound at least 50 cm high, into which the durian is transplanted. Anaerobic fermentation of green manure, particularly using fresh chicken manure, will eradicate *Phytophthora* and other pathogens, while leaving an active population of beneficial soil microbes and a rich source of nutrients for the young seedling.

The watertable should be at least 80 cm below ground level. This can be achieved by planting on a mound 50–60 cm above ground level in lowlands such as the Mekong Delta, or 30–40 cm above ground level elsewhere. Mix pelleted or composted chicken manure and lime into the soil before planting. Select strong and healthy saplings grafted onto disease-resistant rootstocks, like the Vietnamese cv. La queo. Do not plant the saplings too deep and ensure the graft is well above the soil line. Drench the transplanted saplings with phosphonate solution around the base of the plant (10 mL of 400 g/L a.i./10 L water).

When establishing an orchard, space trees widely enough (no more than 80–100 trees/ha for most cultivars), and regularly prune to remove branches within 80–100 cm of the ground to provide adequate ventilation, to reduce canopy humidity, and to minimise soil splash into the canopy. Avoid susceptible clonal monocultures and close interplanting, especially with susceptible plants, as uniformly susceptible monocultures provide ideal conditions for epidemic development. Durian interplanted densely with papaya, coconut, or cocoa which act as alternative hosts, may increase the risk of high levels of disease.

An alternative approach to orchard establishment is to establish a diverse community of plants that mimics the rainforest habitat in which durian evolved. This approach, a type of garden agroforestry, aims to create a biologically diverse, sustainable and highly profitable farming system (Leakey 1998). As a large tree normally forming the

upper canopy of rainforests, durian is ideally suited to this type of planting as a shade tree for understorey fruit trees, vegetables and medicinal plants. The genetic diversity of these mixed plantings significantly retards the development of explosive epidemics, even if some of the intercrops are susceptible to *Phytophthora*.

Mulching

Durian evolved as a rainforest tree. In rainforests, ectomycorrhizal roots absorb mineral nutrients and water from the organic-matter-rich leaf litter layer in the top 50 cm of the soil. Cultivating durian in orchards with bare soil exposes the surface roots to direct sunlight, kills the mycorrhizal fungi, and depletes the biological activity, nutrient availability and health of the topsoil. Irrigation of bare soils under direct sunlight creates a baked crust that inhibits water absorption, forms temporary ponds of water that stimulate sporangial development and zoospore release, and facilitates rainsplash dissemination of *Phytophthora* inoculum.

To recreate the litter layer, especially during orchard establishment, mulch the soil surface under the drip zone of the tree with straw and manure. Mulching encourages mycorrhizal root development, improves soil microbial activity and soil health, suppresses *Phytophthora* and other pathogens and weeds, and improves soil moisture retention in the dry season (Chapter 7.3).

Fresh straw may need to be applied regularly, depending on the local conditions. In the humid wet tropics, such as in north Queensland, the straw decomposes within a few weeks and should be reapplied frequently. In the monsoonal tropics, straw applied toward the end of the rainy season will persist well into the dry season, providing adequate protection for the mycorrhizal roots. Irrigation, whether by spray, drip or flood, can be applied without disturbing the mulch layer, which will also reduce evaporative water loss. During the wet season, it may be wise to clear the mulch from immediately around the base of the trunk to prevent excess moisture persisting directly around the trunk, as this may encourage canker development.

Water and Nutrient Management

Irrigation may be required in environments with a protracted dry season. Spray or drip irrigation is preferred to flood irrigation, with any spray nozzles directed away from the trunk, so that the drip zone, but not the trunk, is wetted. Water that might come from a source at risk of contamination with

Phytophthora should not be used for irrigation. Apply a straw or leaf mulch to cover the ground around the durian tree in the dry season, to reduce water loss from the topsoil.

Organic fertilisers, especially composted chicken manure, are preferred to inorganic fertilisers, as there is evidence that excess inorganic nitrogen increases the risk of phytophthora canker and root rot (Chapter 7.2). Potash fertilisers (supplying potassium) added one month before fruit harvest will prevent the development of 'wet core' and improve fruit quality.

Paclobutrazol, or manipulation of soil water deficits during the rainy season using plastic mulch (Figure 8.1.6) to induce flowering, should be used carefully and not every year. This will avoid stressing the trees.

Harvesting

Once a fruit becomes infected, it takes only about 4 days for it to become completely colonised by *Phytophthora* and then forms an abundant source of inoculum. Regular harvesting and removal of infected fruit reduces the amount of inoculum when fruits are ripening, usually in the rainy season. Remove and bury infected fruit (see below). Fruit should ideally be harvested only when they are still on the tree, and not from the ground. Avoid contact with soil and damage to ripe fruit, as this causes postharvest rot (Chapter 8.6).

Orchard Hygiene and Fruit Disposal

During pruning and harvesting, tools should be disinfected with a quaternary ammonium detergent before they are used on the next tree. Avoid moving soil between orchards on tyres or footwear by washing boots and equipment with a quaternary ammonium detergent.

Infected fruit is a significant source of *Phytophthora* inoculum and should be removed from the orchard. Piles of rotting fruit are also breeding grounds for flying beetles that are potential vectors of the pathogen (Konam and Guest 2004). When composted, fruit also improves soil health and provides a valuable source of nutrients.

If in some years disease pressure is very high and a lot of fruit rot does occur, it is a good practice to anaerobically ferment infected fruit to prevent further spread of the disease, eradicate inoculum and recycle nutrients. This technique is similar to that described for preparing planting holes. Anaerobic fermentation takes approximately 10

weeks, and could be completed in furrows between the rows of trees. Furrows could be constructed every three or four rows, and filled in continuous rotation. Dig a furrow approximately 50 cm deep between rows of trees, and place the diseased fruit into the furrow. Add green manure (such as legume leaves, cut grass and prunings), fresh chicken manure and a starter culture such as EM. When the furrow is almost full, stamp down to exclude as much air as possible, and cover with 5–10 cm of soil.

Canker Treatment

Stem cankers can cause serious tree decline due to damage to the cambium. Cankers reduce tree vigour and yield. They must be diagnosed promptly and accurately for IDM to be successful, and to prevent tree deaths. Once diagnosed, the bark on the surface of cankers should be scraped back and painted with a copper fungicide such as Bordeaux mixture. Ridomil Plus may be used as an alternative, but it is more expensive. The most effective long-term control of canker is achieved through trunk injection of phosphonate.

Trunk Injection of Phosphonate

Potassium salts of phosphorous acid, neutralised to pH 6.5–7.0, and injected into the trunks of trees, give outstanding control of canker and fruit rot (Chapters 8.5 and 8.6). Potassium phosphonate is available under many brand names including Fosject, Foli-R-Fos, Agri-Fos Supa and Phos-Acid. Concentrations of 200 g/L, 400 g/L and 600 g/L a.i. are available. All these concentrations may be injected. The optimal dose for mature durian trees is two or three injections of 16 g a.i. potassium phosphonate annually (depending on the size of the tree and the disease pressure), applied during leaf flush. In mature Vietnamese orchards, trees should be trunk-injected with phosphonate (40 mL of phosphonate 400 g/L a.i.) twice in the first year. As the disease pressure decreases with improved orchard management and the adoption of IDM, injections may be reduced to once a year.

Trunk injection involves drilling a hole 6.5 mm in diameter and 40 mm deep with a sharp drill, about 50 cm from the base of the trunk. Modified veterinary syringes do not work as well on durians as on avocado. Chemjet® injectors (<<http://www.chemjet.com.au/>>) hold 20 mL of phosphonate solution, requiring three or four holes drilled evenly spaced around the trunk, preferably directly under each main branch. Fill an injector and screw into the hole, without pushing, until a clicking sound is heard. Release the spring to allow the

injection to proceed. Under normal conditions injection should take 10–20 minutes. After all the solution has been taken up by the tree, unscrew the injector, rinse first in a quaternary ammonium detergent solution, then in water and refill, and use to inject the next tree. Injectors should be dismantled and thoroughly washed in clean, soapy water at the end of each day.

The Sidewinder® (<<http://www.treeinjectors.com/>>) drills and injects the trunk in one operation, and although it is more expensive, may be practical in large orchards where labour costs are relatively high. Inject trees in the morning, as uptake slows significantly in the afternoon. Care must be taken with high-pressure, trunk-injection systems, as durian trees are prone to splitting of their bark.

Benefit–Cost Analysis

The total cost of phosphonate trunk injection includes the cost of injectors, phosphonate and labour. Chemjet® injectors retail for approximately USD5 each, but last for several years if properly maintained. An average-size, mature durian tree requires 80 mL (four 20 mL injectors) of 200 g/L a.i. formulation, taking up to 30 minutes for complete uptake. A farmer will need at least 20 injectors and one drill for continuous operation, although the cost may be shared by a group of farmers, as each farmer uses them only once or twice a year and they last for several years.

The cost of 32 g a.i. phosphonate required per tree is about USD1 per year (assuming a 400 g/L a.i. formulation costs USD12 per litre). Labour costs vary but, on average, each worker could inject 10 trees per hour. Therefore, the total annual cost of injecting would be about USD2 per tree. If a good-quality durian fruit sells for USD2–5, this means that the cost of injecting a mature tree would be repaid by one extra fruit per tree each year. However, it takes up to 9 years for a tree to become profitable, so the overall cost for the lifetime of an orchard, including the cost of injecting immature trees, might require an extra fruit per tree once the trees are mature.

Assuming an average loss of 20% due to *Phytophthora* and a typical yield of 80 kg, disease control would raise the yield to 100 kg per tree, an increase of 20 kg. At USD2 a fruit, disease control through trunk injection yields a net benefit of USD40 for a cost of USD2. This is a conservative estimate that does not include the savings of not having to replace trees that would otherwise have been killed by canker. The cost of other inputs also varies, and should include the cost of chicken manure, straw mulch and orchard hygiene.

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9 Conclusions and a Vision for Future Research Priorities

André Drenth¹ and David I. Guest²

Abstract

This chapter provides a brief overview of some of the constraints and challenges of trying to develop and implement plant disease-management strategies in short-term international agricultural research projects. General issues relating to focusing on the problem at the local level, developing effective collaborations, finding solutions, overcoming hurdles to adoption, project planning and management, and the interface between funding bodies and research providers are canvassed.

Introduction

Phytophthora diseases cause significant reductions in the yield and quality of food, medicinal and cash crops. In this monograph, some of the common diseases have been discussed in detail and options suggested for sustainable disease management. Although there are solutions for many phytophthora problems, the main challenge is not further basic research, but the adaptation, delivery, implementation, and adoption throughout the region of disease-management strategies that are already available.

Millions of smallholders throughout Southeast Asia could benefit from an enhanced capability to recognise disease problems and implement effective disease-management practices. However, the extremely large numbers of individual growers with diverse personal goals and motivations and a wide range of cultures and languages, together with poorly resourced extension services, make filling this gap a very challenging task.

The Phytophthora Problem

It is clear that *Phytophthora* pathogens can cause many different diseases in many Southeast Asian crops. Phytophthora diseases are difficult to control in the tropics because of the presence of susceptible plant tissues of many different host-plant species and environmental conditions that are conducive to disease development virtually all year round. Although symptoms may abate in the dry season, there is no real break in the disease cycle and inoculum is present all year round. The presence of pronounced wet seasons also significantly aids *Phytophthora* pathogens in their spread and ability to infect susceptible host tissue. The control of these diseases is therefore difficult and an ongoing concern, and there are very few, if any, so-called 'silver bullets' that will solve all the disease problems in a sustainable way. Plant pathologists have long realised that they should use a combination of tools, such as disease-free planting material, orchard management, fertiliser application, disease resistance, fungicides and phosphonate, in an integrated manner if they are to make any significant progress in phytophthora disease management in the tropics. Most smallholders have limited capital or access to credit, further constraining their ability to implement the proposed disease-control methods.

Phytophthora diseases are common and widespread in temperate regions, and have typically been investigated in great detail over long

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periods. This has led to the development of tried-and-tested disease-management options that are implemented and maintained. Unfortunately, this is not the case for phytophthora diseases in the tropics. In tropical areas, a lot less is known about the species involved, the disease cycle and the availability of resistant plant material, and there have been few systematic studies to test and evaluate different disease-management practices. Therefore, the first hurdle to overcome is a technology gap of practical and cost-effective disease-control methods developed and implemented in the tropics.

Working in the tropics, one is continually exposed to comments such as 'we tried this and it did not work' and 'this treatment is very effective'. Further investigation all too often reveals that the statements are not based on statistically rigorous field data. Without knowing exact yield, quality and disease losses it is hard to accurately quantify the effect of different disease-management practices. Therefore, the second hurdle encountered is a shortage of comparative field data, which seriously hampers making choices between different disease-control methods.

The third hurdle is linked to this; it is the lack of baseline data against which the effectiveness of newly introduced disease-management strategies can be compared.

Phytophthora in Southeast Asia

As part of the ACIAR projects that contributed results for this monograph, a workshop was held in Chiang Mai in November 2002, supported by the Thailand Department of Agriculture, ACIAR and the ATSE Crawford Fund. This workshop was the first ever regional meeting on phytophthora in Southeast Asia and provided an excellent networking opportunity for all involved. The aims of the meeting were:

- to review information on the occurrence, impact, species diversity and management of *Phytophthora* pathogens in Southeast Asia and make recommendations for future research
- to review the aetiology and management of fruit rot, patch canker and dieback of durian (*Durio* spp.) caused by *Phytophthora*
- to provide recommendations for the integrated management of phytophthora disease, using durian as a case study.

Based on the field visits, research, field experiments, discussion and the outcomes of the aforementioned workshop towards the end of the project, a number of overall conclusions were reached (Table 9.1).

Table 9.2 lists, in the left-hand column, the needs identified in the original project documentation and

Table 9.1 *Phytophthora* in Southeast Asia: critical issues and solutions identified by two ACIAR projects.

<i>Phytophthora</i> is widespread in Southeast Asia.
Numerous <i>Phytophthora</i> species are involved.
Economic damage is high and needs to be quantified.
<i>Phytophthora</i> epidemics are explosive in favourable weather conditions.
<i>Phytophthora palmivora</i> is the most commonly recorded species and occurs on many hosts.
<i>Phytophthora nicotianae</i> is an important pathogen on many hosts.
<i>Phytophthora cinnamomi</i> is important only in tropical highlands.
<i>Phytophthora infestans</i> is important on potatoes and tomatoes in tropical highlands.
Early detection of symptoms is important for disease control.
The epidemiology of only a few species is understood.
The role of insects as vectors in spread and infection is poorly understood.
Host specificity of <i>Phytophthora palmivora</i> towards the various crops is poorly understood.
The effect of intercropping of hosts susceptible to <i>P. palmivora</i> is poorly understood.
The diversity within <i>Phytophthora palmivora</i> and its centre of origin is unknown.
Some serious pathogens, such as <i>Phytophthora megakarya</i> and <i>P. ramorum</i> , have not been detected in Southeast Asia.
There are numerous disease problems in Southeast Asia on a wide range of minor crops which may be caused by <i>Phytophthora</i> and are in need of further investigation.
There is a need for development and implementation of integrated disease-control methods for a wide range of disease problems in the region.

during the course of both ACIAR projects. Activities to provide a solution to those needs are listed in the right-hand column.

Future Research Priorities Concerning *Phytophthora*

Although both projects addressed some of the needs outlined in Table 9.2, there is clearly an enormous need to tackle some of the most devastating diseases in a wide range of crops in the tropics. In this monograph, durian has been used as an example of how to develop and implement effective integrated disease management practices. While each crop has its own specific problems and needs, the following general advice should aid the setting of research priorities that apply to a wide range of crops:

- encourage regional and international collaboration to detect and identify sources of resistance towards phytophthora diseases

- focus on screening and selection programs to identify germplasm of crops suitable for the each growing region
- critically evaluate the aims of breeding programs that too often focus strongly on yield, ignoring the reality that yield potential is hardly ever a constraint for the smallholder, whose yields are much more likely to be constrained by the lack of inputs and high levels of diseases and pests
- need to identify good source of resistance and protection of wild germplasm of crop plant species and their relatives
- need for robust tests for disease-resistance screening of local materials and breeding lines
- more research is needed on disease complexes such as yield declines and replanting diseases
- need to collect and disseminate comparative field data of food crops and identify constraints to profitability by smallholders.
- attention to nursery hygiene for tree crops

Table 9.2 Needs concerning *Phytophthora* in Southeast Asia and solutions provided by ACIAR projects PHT/1995/134 and PHT/1996/193.

Needs	Solutions
Training in all aspects of <i>Phytophthora</i> biology and disease control	Start-up workshop Hands-on training Field trips Field experiments Workshop in Chiang Mai Practical guide to detection and identification of <i>Phytophthora</i>
Training in development and implementation of disease management practices	Through nursery and field visits, field experiments, extension activities and uptake of recommendations by farmers, a significant improvement of disease control in durians has been achieved.
Focus on integrated disease management	Through discussions, farmer field visits, workshops and extension activities both projects had a strong practical focus on implementation of disease-control strategies.
Improve accessibility of information	This monograph reviews a large proportion of the information from the collaborating countries and makes it available to all project collaborators and others. Lists of recent theses on <i>Phytophthora</i> submitted in Thailand have been collated.
Accurate species identification	Close to 500 species identifications were performed in the survey project.
Occurrence of <i>Phytophthora</i> species in Southeast Asia	Tabulation of <i>Phytophthora</i> records in the country reports in this monograph.
Disease records, reference strains and collections	Disease records published as part of this monograph and strains lodged in BRIP Brisbane and information made available to relevant country.
Coordination of government and international research institute programs	Both ACIAR projects involved a large number of collaborators from many different organisations working on the same problems. This unique networking opportunity forms the basis for further collaboration in the future.
Networking	A website established at the University of Melbourne for the durian project. Regular contact by email between the participants in the various countries.
<i>Phytophthora</i> management in forests	Solutions are needed but they were not covered in these projects.

- projects on the development of integrated disease and pest management
- focus on development and adoption of appropriate technologies based on sound principles of integrated disease management
- follow up on reports of emerging phytophthora diseases in crops including longan, mango, mangosteen and coffee.

Thoughts on the Challenges of International Research Projects

In order to improve the uptake of research findings and make the outcomes of international collaborative projects available as widely as possible a clear focus is needed. Projects should:

- clearly define practical problems at the local level
- foster partnerships and establish an effective and experienced project team committed to finding solutions to the problem
- take into account the profitability and risk exposure of local growers
- find effective and realistic solutions which address the real need and the real problem
- involve all players in the chain of production, processing, transport and marketing in the form of a stakeholder platform
- focus on implementation and adoption of the research findings and solutions to the target group
- ensure the collection of comparative field data to form a foundation to build upon
- establish benchmarks for performance comparisons
- include long-term training that enables the formation of enduring partnerships
- deliver long-term benefits.

The success of any project is highly dependent on how well the problem is defined at the start of the work. Funding agencies, policy makers and governments need to make difficult choices about resource allocation to priorities and problems they want to address. With a plethora of problems in agricultural production it would make sense to focus on the problems that cause most significant losses. This immediately leads to the question of who decides what is significant and how they define it. Defining priorities can further complicate decision-making. Priorities can be defined in economic terms, food-security terms, impacts on smallholders, or long-term development goals for the country, among others.

An important question to consider is: What difference will it make if the team successfully conducts the project, implements the findings and

gets good adoption by the local growers? In such an analysis one needs to assess positives and negatives. Hence, stakeholders should evaluate projects on the basis of the potential positive impacts they may bring, and carefully weigh these up against negative impacts.

Finding solutions to problems within the constraint of available resources and time is essential. It is important that projects be set up and planned in such a way that they are realistic, achievable and provide a foundation for future improvement. It is also important to consider if the solution can be widely applied to other crops and regions. There is always a temptation to conduct projects in a number of regions simultaneously, but it may be wiser and more efficient to show that the solution works in one region before attempting to implement it elsewhere.

Once the problem is defined, the search for an effective project team with a track record of delivery of outcomes is needed to implement the solutions. There has to be a reappraisal of the value of spending scarce research dollars on fashionable, highly advanced and expensive research that is typically never implemented due to its high cost and marginal benefit. Priority should be given to implementing practical solutions based on existing technology. Researchers do not need to pursue glamorous technological solutions if simple and low-key technological solutions are effective. The delivery of the research outcomes that benefit large numbers of smallholders should be a high priority.

It is important to obtain field data on an ongoing basis. Without field data – simply defined here as yield, quality, disease loss, price, price of inputs and farmer income and farming profitability – project teams cannot measure long-term improvements in production, quality and profitability. Hence, it is important to work as colleagues to capture this information on an ongoing basis to provide a benchmark against which to measure gains. Experimental scientists working in tropical countries need to have the ability and confidence to conceptualise, design, execute and interpret field experiments.

There has to be a significant improvement in agricultural income and profitability to bridge the gap between the countryside and the city in many developing countries. Donors and project teams have to be careful not to burden people in developing countries through overly optimistic expectations of biotechnology or notions of farming with no inputs, sometimes confused with organic farming. In order to stop land degradation and the

ever-increasing land areas needed to feed the world's population, a rapid and sustainable increase in productivity and profitability is needed from the agricultural land already under cultivation.

Any organisation commissioning international agricultural research projects involving developing countries should have a strong focus on fostering implementation of the findings. Without implementation and adoption of the outcomes, research into disease management is futile. However, donors have to be extremely careful that they support initiatives that lead to implementation of the right solution, and in order to find the best solutions they must foster partnerships that can deliver what the country and industry need.

The training through partnership with scientists from developing countries was an important aspect of our projects. All-round training in science coupled with hands-on field experience is needed now more than ever. In an effort to make a significant contribution to one of the main challenges of the 21st century — food supply and food security — this

generation needs to train the next generation of scientists and provide them with hands-on experience in complex technical or biological areas.

In order for young scientists from developing countries to become an asset to their own country, they often need a mentor with accumulated practical experience in science and field experimentation, and a capability to implement effective solutions. Ideally, agricultural research training should include hands-on training in the form of internships with experienced researchers or mentors, over a long period. In order to facilitate and support such an endeavour, a partnership program may be needed whereby research organisations involved in the same research field form a bilateral link and exchange staff and students to form effective and long-term partnerships. It is important that project leaders have hands-on experience of working in the facilities and with the extension staff of organisations in developing countries, so as to fully understand the challenges of working in a resource-limited environment.

Appendix

Table of *Phytophthora* pathogens and hosts in Southeast Asia

Table of *Phytophthora* pathogens and hosts in Southeast Asia

<i>Phytophthora</i> species	Host	Disease ^a	Country	Pages referred to in this monograph
<i>P. arecae</i> (Coleman) Pethybridge	Coconut (<i>Cocos nucifera</i> L.)	Bud rot and nut fall Bud rot and fruit rot	Indonesia Philippines	78 117
<i>P. botryosa</i> Chee	Rubber (<i>Hevea brasiliensis</i> Meull. Arg.)	Leaf fall and pod rot Black stripe Leaf fall and black stripe	Malaysia Thailand Vietnam	61, 62 79 88
<i>P. cactorum</i> (Lebert and Cohn) Schröeter (= <i>P. omnitrova</i> de Bary)	Apple (<i>Malus pumila</i> Mill.) Avocado (<i>Persea americana</i> Mill.) Cocoa (<i>Theobroma cacao</i> L.) Plum (<i>Prunus salicina</i> Lindl.)	Collar rot Root rot and stem canker Oriental cocoa pod disease Black spot and fruit rot	Indonesia Indonesia Philippines Vietnam	71 71 91 84, 87, 88
<i>P. capsici</i> Leonian (= <i>P. palmivora</i> MF4)	Black pepper (<i>Piper nigrum</i> L.)	Foot rot Foot rot Foot rot Root and stem rot Fruit rot Crown and root rot –	Malaysia Indonesia Vietnam Thailand Indonesia Philippines Thailand	60, 66 70, 132–134, 171–173 84 77, 78 71 91 138
<i>P. cinnamomi</i> Rands	Rubber (<i>Hevea brasiliensis</i> Meull. Arg.) Cinchona (<i>Cinchona ledgeriana</i> Moens and <i>C. succirubra</i> Pav. Ex. Klotzsch Cinnamon (<i>Cinnamomum burmannii</i> (Nees) Blume) Pineapple (<i>Ananas comosus</i> (L.) Cinchona (<i>Cinchona calisaya</i> Wedd.)	Root rot and dieback Bark canker Bark canker Heart rot Seedling dieback	Malaysia Indonesia Indonesia Vietnam Indonesia	61, 62 70, 71 70, 71 84–86 71
<i>P. citricola</i> Sawada	Citrus (<i>Citrus</i> spp.)	Foot rot, gummosis and brown rot Gummosis and fruit rot Crown and root rot Crown and root rot	Philippines Vietnam Philippines Philippines	91 86 91 91
<i>P. colocasiae</i> Raciborski	Betel vine (<i>Piper betle</i> L.) Yam/taro (<i>Colocasia esculenta</i> (L.) Schott.)	– – Leaf blight – –	Malaysia Malaysia Indonesia Vietnam Philippines	61–62 61–62 71 84–85 91
<i>P. heveae</i> Thompson	Cocoa (<i>Theobroma cacao</i> L.) Rubber (<i>Hevea brasiliensis</i> Meull. Arg.) Santol (<i>Sandoricum koetjape</i> Merr.)	Pod disease – Crown and root rot	Malaysia Malaysia Philippines	61–62 61–62 91
<i>P. infestans</i> (Mont.) de Bary	Potato (<i>Solanum tuberosum</i> L.) Tomato (<i>Lycopersicon esculentum</i> Mill.)	Late blight Late blight – Late blight Late blight – Late blight	Malaysia Indonesia Thailand Philippines Vietnam Malaysia Indonesia	61–62 71 78 91 83–84 61–62 71

(Cont'd) Table of *Phytophthora* pathogens and hosts in Southeast Asia

<i>Phytophthora</i> species	Host	Disease ^a	Country	Pages referred to in this monograph
<i>P. meadii</i> McRae	Betel (<i>Piper betle</i> L.) Cocoa (<i>Theobroma cacao</i> L.) Rubber (<i>Hevea brasiliensis</i> Meull. Arg.)	- Pods Black thread - -	Malaysia Malaysia Philippines Malaysia Thailand	61 63 91 61, 137-139 78
<i>P. nicotianae</i> Breda de Haan = <i>P. parasitica</i> Dastur = <i>P. nicotianae</i> var. <i>nicotianae</i> = <i>P. nicotianae</i> Breda de Haan var. <i>parasitica</i> (Dastur) G.M. Waterhouse = <i>P. melongenae</i> Sawada	Betel (<i>Piper betle</i> L.) Brinjal (<i>Solanum melongena</i> L.) Citrus (<i>Citrus</i> spp.) Coconut (<i>Cocos nucifera</i> L.) Durian (<i>Durio zibethinus</i> Murr.) Eggplant (<i>Solanum melongena</i> L.) Guava (<i>Psidium guajava</i> L.) Longan (<i>Euphoria longana</i> Lam.) Orchid (<i>Vanda</i> spp.) Papaya (<i>Carica papaya</i> L.) Passionfruit (<i>Passiflora edulis</i> Sims.) Pepper (<i>Piper nigrum</i> L.) Periwinkle (<i>Vinca rosea</i> L.) Pineapple (<i>Ananas comosus</i> (L.) Rosella (<i>Hibiscus sabdarifra</i> L.) Scarlet sage (<i>Salvia splendens</i> Sellow ex Roemer & J.A. Schultes) Strawberry (<i>Fragaria</i> sp.) Tobacco (<i>Nicotiana tabacum</i> L.) Vanilla (<i>Vanilla planifolia</i> Andrews) Watermelon (<i>Citrullus lanatus</i> Schrad.)	- - Foot and root rot Root and fruit rot Stem canker Root rot and gummosis Bud rot and nut fall Root rot and canker Fruit rot Fruit rot Leaf blight Root rot - Blight and fruit rot Fruit, collar and root diseases - Root and stem rot Crown and root rot - - - - Heart rot and wilt Heart rot and root rot Heart rot Wilt Black foot rot - - Black shank Black shank Pod rot Rot	Malaysia Malaysia Malaysia Thailand Vietnam Philippines Indonesia Thailand Malaysia Philippines Indonesia Malaysia Thailand Malaysia Philippines Malaysia Thailand Philippines Malaysia Thailand Philippines Indonesia Vietnam Malaysia Indonesia Malaysia Malaysia Indonesia Vietnam Indonesia Philippines	61-62 61-62 61, 62 77-78 84, 86 91 73 78 143 91-92 71 61 78 61 91 61 61 77-78 91 66 132 61 78 91-92 71 84-86 61-62 71 61-62 61 71 71 84 84 71 91

(Cont'd) Table of *Phytophthora* pathogens and hosts in Southeast Asia

<i>Phytophthora</i> species	Host	Disease ^a	Country	Pages referred to in this monograph
<i>Phytophthora palmivora</i> Butler (syn. <i>P. fiberei</i> Maubl.)	Avocado (<i>Persa americana</i> Mill.)	Crown and root rot	Philippines	91
	Citrus (<i>Citrus</i> spp.)	Blight	Philippines	91
Cocoa (<i>Theobroma cacao</i> L.)		Gummosis	Indonesia	71
		Black pod	Malaysia	61, 62
Coconut (<i>Cocos nucifera</i> L.)		Pod rot, stem and cushion cankers, leaf, chupon and seedling blights and sudden death	Indonesia	11, 71, 72
		Black rot and stem canker	Philippines	91
		Pod rot	Thailand	77, 78
		Fruit rot	Vietnam	84
		Bud rot and premature nut fall	Indonesia	73
		Bud rot and premature nut fall	Philippines	91, 116, 124
		Nut drop	Thailand	78
		Stem canker	Vietnam	84
		Trunk and root rot, patch canker	Malaysia	60-62
		Patch canker	Indonesia	71
Durian (<i>Durio zibethinus</i> Murr.)		Trunk canker, root, stem and fruit rot	Thailand	53, 77
		Crown and root rot	Philippines	91
		Root and fruit rot, stem canker and leaf blight	Vietnam	84
		Crown and root rot		84, 87
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Root rot	Thailand	78
		-	Thailand	78
Jackfruit (<i>Artocarpus</i> spp.)		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Root rot	Thailand	78
		-	Thailand	78
		Crown and root rot	Philippines	91
		Crown and root rot	Philippines	91
		Black rot	Philippines	91
Mango (<i>Mangifera indica</i> L.)		-	Malaysia	61, 62
		Crown and root rot	Indonesia	71
		Crown and root rot	Indonesia	71
		Black rot	Malaysia	61, 62
		Root, collar and fruit rot	Malaysia	61, 62
		Fruit rot	Philippines	91
		Root and fruit rot	Indonesia	71
		Sudden death	Malaysia	61
		Root rot	Thailand	77, 78
		-	Thailand	78
Kamansi (<i>Artocarpus camansi</i> Blanco)		Root rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Lanzones (<i>Lansium domesticum</i> Corréa)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Longan (<i>Euphoria longana</i> Lam.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Mangosteen (<i>Garcinia mangostana</i> L.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Neem (<i>Azadirachta indica</i> A. Juss.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Orchid (<i>Phalaenopsis</i> spp.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Papaya (<i>Carica papaya</i> L.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Pepper (<i>Piper nigrum</i> L.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
Pineapple (<i>Ananas comosus</i> (L.)		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91
		Black rot	Philippines	91

(Cont'd) Table of *Phytophthora* pathogens and hosts in Southeast Asia

<i>Phytophthora</i> species	Host	Disease ^a	Country	Pages referred to in this monograph
<i>Phytophthora palmivora</i> Butler (syn. <i>P. faberi</i> Maubl.) - cont'd	Cinchona (<i>Cinchona calisaya</i> Wedd.)	Seedling blight	Philippines	91, 92
	Rubber (<i>Hevea brasiliensis</i> Meull. Arg.)	Leaf fall and black stripe	Thailand	78
		Leaf fall and black stripe	Vietnam	88
		Canker and black rot of fruit	Philippines	91
		Black stripe and patch canker	Malaysia	60, 62
		Black stripe and leaf fall	Indonesia	71
	Soursop (<i>Annona muricata</i> L.)	Crown and root rot	Philippines	91
	Tamarind (<i>Tamarindus indica</i> L.)	Crown and root rot	Philippines	91
	Vanilla (<i>Vanilla</i> spp.)	Black rot	Thailand	78
	<i>P. phaseoli</i> Thaxt.	Santol (<i>Sandoricum koejape</i> Merr.)	Leaf blight	Philippines
<i>Phytophthora</i> sp.	Avocado (<i>Persea americana</i> Mill.)	-	Malaysia	61
	Citrus (<i>Citrus</i> spp.)	Gummosis and fruit rot	Vietnam	84
	Longan (<i>Euphoria longana</i> Lam.)	Fruit rot and leaf blight	Vietnam	84
	Pepper (<i>Piper nigrum</i> L.)	-	Malaysia	62
	Rubber (<i>Hevea brasiliensis</i> Meull. Arg.)	-	Malaysia	62
		Stem canker	Vietnam	84
	Ziziphus (<i>Zizyphus mauritania</i>)	Fruit rot	Vietnam	84

^a - = no specific disease identified.