Australian Government

Biosecurity Australia

Final Import Risk Analysis Report for the Importation of Cavendish Bananas from the Philippines

Part C

November 2008

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Cover image: Emerging banana bunch showing bracts, flowers and fingers (Photo: D Peasley)

This import risk analysis has been released by the Chief Executive of Biosecurity Australia.

Stakeholders have 30 days from the publication of this document to lodge an appeal in writing with the Import Risk Analysis Appeals Panel – a body independent of Biosecurity Australia – on one or both of the following grounds:

> There was a significant deviation from the process set out in the Import Risk Analysis Handbook (BA 2003) that adversely affected the interests of a stakeholder.
>
> A significant body of scientific information relevant to the outcome of the IRA was not considered.

In lodging appeals, stakeholders must give reasons for their appeal.

Appeals should be submitted to:

> IRAAP Secretariat
> Corporate Policy Division
> Department of Agriculture, Fisheries and Forestry
> GPO Box 858
> CANBERRA ACT 2601

Facsimile: +61 2 6272 5926
E-mail: IRAAP@daff.gov.au

Further details of the appeal process are provided in the Handbook (BA 2003).

Contents

Appendix 1. Restrictions on movement of bananas within Australia **1**

Appendix 2. Growing conditions for bananas in the Philippines and Australia **5**

Appendix 3. Pathogen categorisation **14**

Appendix 4. Arthropod categorisation **21**

Appendix 5. Moko **35**

Appendix 6. Black Sigatoka **69**

Appendix 7. Freckle **89**

Appendix 8. Banana bract mosaic virus **107**

Appendix 9. Banana bunchy top virus **119**

Appendix 10. Fruit flies **131**

Appendix 11. Armoured scales **135**

11.1 *Aspidiotus coryphae* 138
11.2 *Aspidiotus excisus* 139
11.3 *Pinnaspis musae* 141
11.4 *Abgrallaspis cyanophylli* 142
11.5 *Hemiberlesia palmae* 144
11.6 *Pseudaulacaspis cockerelli* 145
11.7 *Selenaspidus articulatus* 147

Appendix 12. Mealybugs **149**

12.1 *Dysmicoccus neobrevipes* 150
12.2 *Nipaecoccus nipae* 154
12.3 *Pseudococcus jackbeardsleyi* 156
12.4 *Planococcus minor* 159

Appendix 13. Spider mites **161**

13.1 *Oligonychus orthius* 164
13.2 *Oligonychus velascoi* 165
13.3 *Raoiella indica* 167
13.4 *Tetranychus piercei* 169
13.5 *Tetranychus marianae* 173

Appendix 14. Weevils **175**

Appendix 15. Thrips **177**

Appendix 1. Restrictions on movement of bananas within Australia

Movement of banana fruit and planting material within Australia is subject to quarantine restrictions. Queensland, New South Wales, Western Australia and the Northern Territory have quarantine legislation to control the movement of bananas and banana material intra and interstate.

Fruit flies are of concern to all Australian states and territories. However, with respect to fruit flies, banana fruit may be moved into any state or territory as long as they are certified under the nationally approved interstate certification assurance (ICA) procedures that the fruit is in a hard green (ICA-06) condition (QDPI 2000). This ICA requires the following:

- Fruit of the variety Cavendish only.
- The banana flesh is hard and not flexible, the skin is green and shows no yellow colouration except for areas towards the flower end of the fruit, in which the sun has bleached the skin to a yellow to white colour. The flesh beneath is hard.
- No single banana or banana on an outside whorl of a hand or cluster (except a wing banana or distorted banana) has a diameter that exceeds 42 mm when measured at right angles to the curvature of the fruit at a point one third from its flower end.
- The skin must be unbroken.

Due to concerns over black Sigatoka, *banana bunchy top virus* (BBTV), Panama disease and various soilborne pests, all states and the Northern Territory restrict the movement of banana planting material.

Below is a brief summary of the intra- and interstate requirements for diseases that have been associated with bananas.

Queensland

Six pest quarantine areas for banana pests have been established in Queensland, and movement of banana plants and their products, including fruit, may be controlled within and between these areas. Queensland legislation provides a contingency for outbreaks of quarantine pests. Black Sigatoka, bacterial wilts (blood disease and Moko), *banana bract mosaic virus* (BBrMV) and BBTV are among the notifiable pests, and legislation allows for the restriction to the movement of banana fruit, plant material and other if an outbreak is recorded. An inspector’s approval would be required to remove banana fruit and other plant material from an outbreak area.

Intrastate restrictions may be applied to the movement of banana fruit within Queensland. Provisions may also be invoked for any unforseen detections of exotic diseases and may be applied to an affected pest site. A ‘pest site’ is defined as an area within approximately 50 kilometres of a place where a banana pest has been found. The chief executive of the state officially declares the boundaries of a pest quarantine area. A declared pest area would be subject to intrastate restrictions relating, as necessary, to the movement of bananas originating from such an area. Restrictions would also apply to the movement of bananas into Queensland from other areas if any of the above diseases or other pests of quarantine concern to Queensland would be recorded there.

Currently there are no restrictions on the movement of hard green banana fruit into Queensland (QDPI 2000). As Queensland produces approximately 90% of Australia’s bananas, the quantity of bananas entering Queensland from other states is usually very low; hence, the risk of entry of pests on the fruit pathway is also considered low.

Further information on the Queensland requirements for intra- and interstate movement of banana plants and plant products is available in *Plant Protection Regulation 2002* of the *Plant Protection Act 1989*.

New South Wales

New South Wales, under Proclamation – P172, proclaims certain pests and diseases as notifiable under the *Plant Diseases Act 1924.* The list includes the following diseases affecting banana: black Sigatoka, Panama disease (tropical race 4), BBTV, BBrMV, freckle, blood disease and Moko.

Following the outbreak of black Sigatoka in the Tully district of north Queensland in April 2001, New South Wales enacted legislation (Proclamation – P119) in January 2002 to control movement of banana fruit and packaging materials from the Tully district.

Under this legislation, bananas grown or packed in parts of Queensland north of the 22nd parallel of south latitude could not be taken into the New South Wales Banana Protection Area. At the time, this consisted of 17 LGAs in northern New South Wales – Ballina, Bellingen, Byron Bay, Coffs Harbour, Copmanhurst, Grafton, Great Lakes, Greater Taree, Hastings, Lismore, Kempsey, Kyogle, MacLean, Nambucca, Pristine Waters, Richmond Valley and Tweed. However, bananas from parts of Queensland north of the 22nd parallel of south latitude could be moved to other parts of New South Wales, subject to the following conditions (if approved by the New South Wales Department of Primary Industries):

- Fruit must be commercially packed
- Packaging must be free of any banana leaf material or trash
- Packaging must be clearly marked with the Interstate Produce Number (IPN) under the Interstate Certification Scheme.

Only bananas originating from areas of Queensland south of the 22nd parallel can be taken into any part of NSW without restrictions, subject to adequate labelling.

In February 2005, the ban restricting the movement of banana fruit from Tully into the New South Wales Banana Protection Area was revoked after the successful eradication of black Sigatoka from the Tully district of north Queensland (Proclamation – P119R).

Orders 77/06/01 and 77/06/02, gazetted 10 October 2007, for the treatment and eradication of banana diseases and pests within the NSW banana protected area:

- require the destruction of Musaceae plants and banana aphids; and
- regulates the movement of Cavendish and Panama disease resistant Musaceae planting material.

Western Australia

Banana fruit is allowed to enter Western Australia (WA) from other areas of Australia. However, it is subject to various regulations. These include:

As a precaution against the fruit flies *B. tryoni*, *B. musae* and *B. neohumuralis,* banana fruit must certified by the exporting state or territory's quarantine authority or under an approved quality assurance arrangement as either:

a) Dimethoate dipped or sprayed; or
b) fenthion dipped or sprayed; or
c) methyl bromide fumigated; or
d) cold treated; or
e) as being harvested in a green mature condition.

As an alternative to (d) banana fruit will be accepted if in a green mature condition on arrival in WA.

All consignments must be free of live pests (including the regional pests *Planococcus minor, Abgrallaspis cyanophylli, Hemiberlesia palmae, Pseudaulacaspis cockerelli* and *Chaetanaphothrips signipennis*), disease symptoms, contaminant seeds, soil and other debris.

Banana fruit imported from within 50 km of a known outbreak of Panama disease tropical race 4 (*Fusarium oxysporum* f. sp. *cubense*) must be verified as free from soil and plant material before being permitted entry into the banana growing areas of Carnarvon, Broome and Kununurra.

Each consignment must be packed in clean new packaging with the commodity type, the commodity producer, packer or agent and the district of production printed on an external surface in letters of not less than 5 mm in height.

Each consignment is inspected on arrival and treated if necessary.

If any plant material other than the permitted commodity or contaminants (including soil or seeds) is found on inspection, the consignment must be held and the contaminants removed or treated, or the goods re-exported or destroyed.

If live insects are detected, the consignment must be held pending identification and may be treated or the goods re-exported or destroyed.

In April 2001, following the outbreak of black Sigatoka in the Tully district of northern Queensland, Western Australia imposed new restrictions on imports of banana fruit from Queensland. At this time Western Australia also developed, under Section 23B of the *Plant Disease Act 1989*, a temporary alternative to Schedule 1 of the *Plant Diseases Regulations 1989.* Bananas grown or packed within 50 km of an occurrence of black Sigatoka, or packed in premises that handled bananas grown within 50 km of an occurrence of black Sigatoka, were prohibited entry into Western Australia, except under conditions approved by Western Australia’s Department of Agriculture.

A risk analysis conducted by Western Australia’s Department of Agriculture subsequently recommended that fruit originating within 50 km of an outbreak of black Sigatoka could be marketed south of the 26th parallel in the State (Kumar et al 2002). Western Australia accepted this recommendation and allowed fruit into Perth and the southern areas in August 2003. In June 2004, the ban restricting the importation of banana fruit from the Tully district of north Queensland into Western Australia (both north and south of the 26th parallel of south latitude) was revoked.

There are no specific measures for the importation of banana fruit for BBTV. However, as the banana aphid (*Pentalonia nigronervosa*) is a vector of the virus, bananas are subject to inspection for this insect. In any case, all consignments of banana fruit entering Western Australia must be free of live pests, disease symptoms, contaminant seeds, soil and other debris.

Northern Territory

Under the provisions of the *Plant Diseases Control Act 1979*, banana fruit grown within 50 km of a known case of black Sigatoka was not permitted entry into the Northern Territory. However, this ban was lifted after the eradication of black Sigatoka in north Queensland.

There are no restrictions on the transport of mature hard green banana fruit into the Northern Territory from other states. However, the movement of plant material is restricted and can only be conducted under permit between states or quarantine areas.

Industry initiative

The Australian banana industry and Plant Health Australia have developed a National Banana Industry Biosecurity Plan (PHA 2004b). The plan aims to deliver a nationally consistent method to identify incursion risks, develop emergency plant priority lists and contingency plans, define roles and responsibilities and conduct regular reviews to maintain effective industry biosecurity measures.

An Emergency Plant Pest Deed has also been signed between the Australian Government, state governments and the peak industry body (ABGC), which carries obligations for each party in regard to preparedness and response.

Appendix 2. Growing conditions for bananas in the Philippines and Australia

This appendix summarises the climatic conditions, topography, soils and production characteristics of the major banana growing areas of Australia, and the Philippine island of Mindanao – from where bananas would be sourced for export to Australia.

Philippines banana industry – growing conditions

Climate

The climate of the Philippines enables production of bananas throughout the year (Figure 2.1; PCARRD 1988). The proposed export area (that is, areas of Davao, Cotabato and Bukidnon provinces on Mindanao Island) is compact, but climate varies between the lowland and highland areas. The production area in Bukidnon is elevated (over 500 m), with cooler and drier conditions providing for slower growing and bunch filling than in the coastal lowlands.

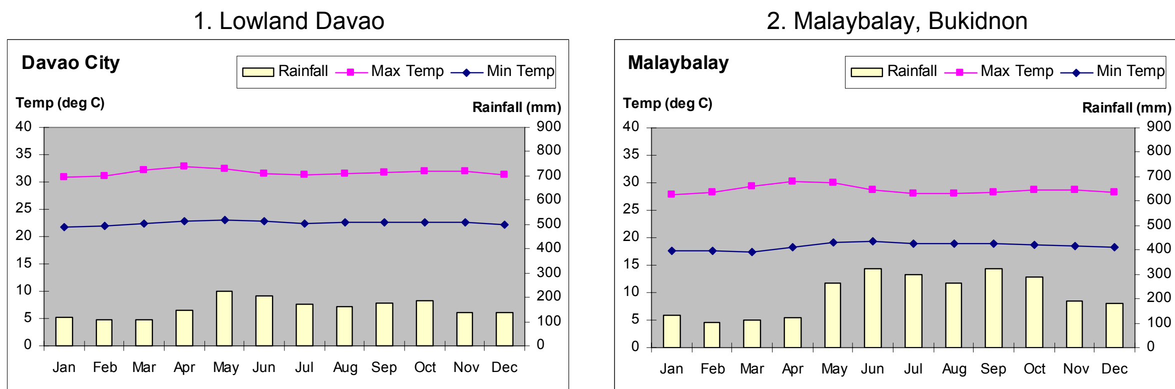


Figure 2.1 Climate representative of banana growing areas in the Philippines

Data source: World Climate (http://www.worldclimate.com)

Topography and soils

Soils are generally heavier in the coastal lowlands. Drainage systems are designed to quickly remove excess water and reduce waterlogging and flooding of plantations (Peasley 2001). The highlands and plateau areas of Bukidnon are located on well-drained basaltic krasnozem soils with variable slopes. Plantations in Bukidnon are not as extensive as those in the coastal lowlands and tend to be separated by gullies or land that is generally too steep for mechanised cultivation.

Australian banana industry – growing conditions

The commercial banana industry in Australia is concentrated on the tropical Queensland coast between Babinda and Cardwell (designated ‘north Queensland’), with the largest production area located in the Tully region. Other banana production areas are South-East Queensland (from Bundaberg to the New South Wales border), northern New South Wales (from the Queensland border to Kempsey), Carnarvon and Kununurra in Western Australia and Darwin in the Northern Territory. The climatic characteristics of each region are summarised in Table 2.1.

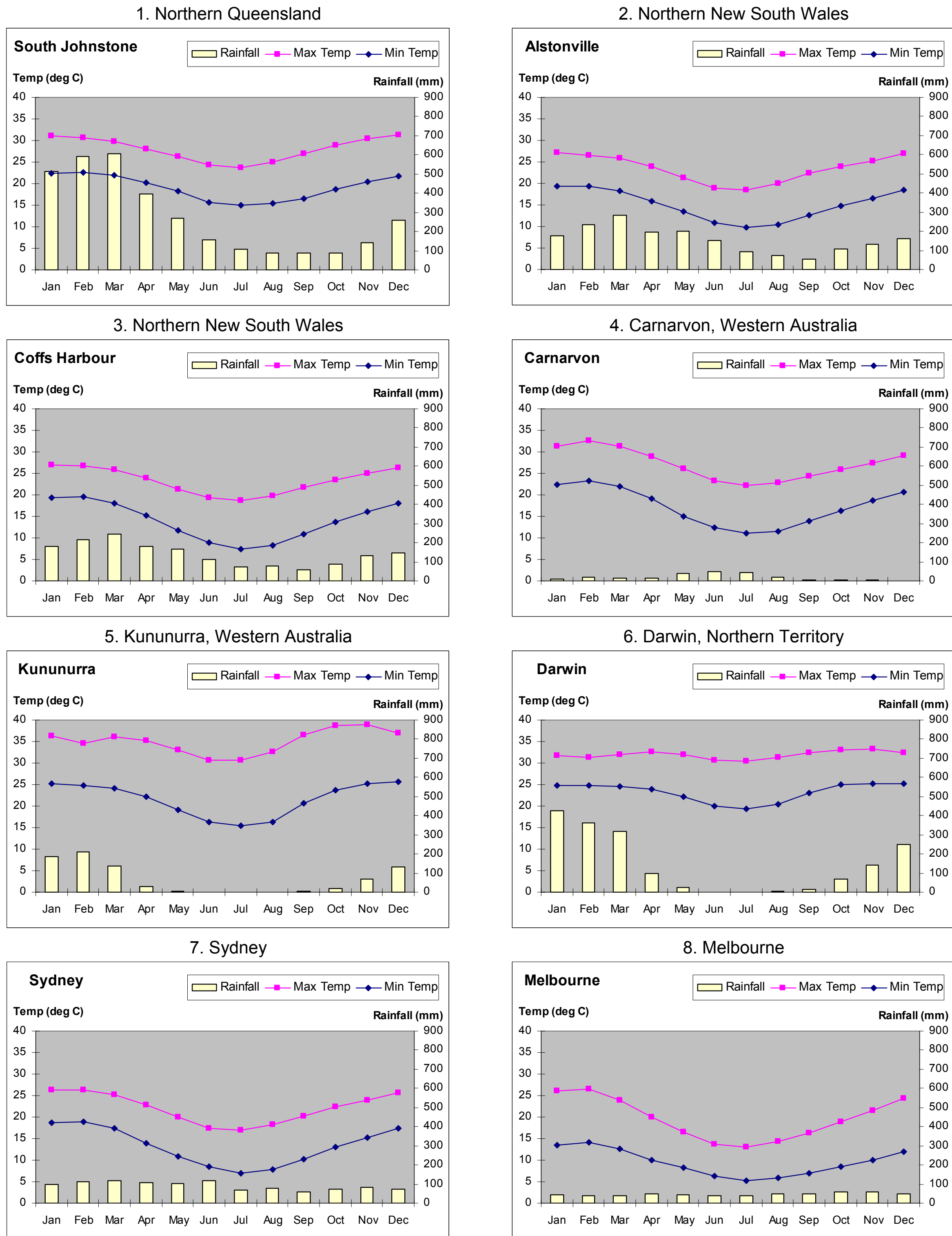


Figure 2.2 Climate in banana growing and non-growing areas in Australia

Data source: Australian Government Bureau of Meteorology (http://www.bom.gov.au)
Graphs 1–6 illustrate banana growing areas and graphs 7 and 8 non-growing areas.

The Australian market is supplied year-round from the six areas of Australia that produce bananas. The rainfall, temperature (Figure 2.2) and topography of these areas are distinct, resulting in a range of

production and management practices, and a range of key pests and diseases. Geographic isolation of each growing area in Australia also provides some protection against the occasional disruption of supply caused by cyclones, storms and pest outbreaks.

Table 2.1 Climatic characteristics of Australia's banana production regions

Banana production area	Climate type	Percent of production[1]
North Queensland	Wet/dry tropical	89.4%
South-East Queensland	Humid subtropical	
Northern New South Wales	Humid subtropical	6.8%
Carnarvon, Western Australia	Semi-arid subtropical	3.1%
Kununurra, Western Australia	Semi-arid monsoon	
Northern Territory	Semi-arid monsoon	0.7%

[1]Percentage based on 2004–05 production figures (ABS 2006; see Part B, Chapter 2)

North Queensland

Climate

The climate in north Queensland is tropical, with a pronounced wet season in summer and early autumn (December–March) when rainfall, under the influence of cyclonic disturbances, can be extreme (Figure 2.2), and a dry season in winter and spring (June–October). To supplement rainfall during the dry season, under-canopy irrigation is required in most banana plantations in order to maintain high levels of production.

Topography and soils

Bananas will grow on a wide range of soil types, but best production requires well-drained soil, preferably a clay loam at least 0.5 m deep (Lindsay et al 2005). Plantations for 99% of bananas grown on the Tully Valley floodplain and 60% of those grown in the Innisfail area are located on light to medium alluvial clays. The remaining 40% of plantations on the undulating slopes of the Innisfail area are on basaltic krasnozem soils (BA 2002c).

Following severe cyclonic or rainfall depressions, the floodplains of the Tully and Innisfail areas are subject to regular inundation and widespread flooding. During this period, soil temperatures are also high, creating highly favourable conditions for the spread of soilborne diseases. In the Tully region, high intensity rainfall, sufficient to cause flooding, occurs on average every second year (Peasley 2006a). In the major flood event of 1999, approximately 70% of banana plantations in the Tully Valley and 10–15% of the Innisfail production area were flooded (BA 2002c). This was regarded as a one-in-25-year event (Peasley 2006a). Production areas free from flooding are considered desirable.

Production and handling systems

North Queensland banana production is made up of 569 farms of varying sizes, with an average size of 18 hectares (ABGC 2005). Most of the holdings are family-operated. The north Queensland production area is characterised by mechanised production, the degree of mechanisation being determined principally by farm size. On large farms, field operations are conducted from vehicles (Figure 2.3), including de-leafing, bagging, pruning, harvesting, weed and pest spraying and monitoring, nematicide application, fertilising and irrigation monitoring.

Bananas are planted on mounded rows to provide drainage for high productivity. Row length is a function of farm size and configuration, as well as of the cost-effective distance for management operations such as harvesting, spraying, monitoring and irrigation. Rows may be up to 800 m long. The inter-row area acts as the drain for excess water from irrigation, or from rainfall and flooding.

The inter-rows are also the sole access for vehicles and these are very muddy during 'wet' periods. During 'dry' periods also, the inter-row is muddy in most plantations due to irrigation and the accumulation of spent banana stems, leaves and organic matter following harvesting, de-leafing and pruning operations. Under such conditions, four-wheel drive vehicles with specially designed tyres are used. Inter-rows are generally travelled at least three times each week by a variety of vehicles, including four-wheel drive motor bikes, purpose-built four-wheel drive bagging machines, tractors, harvesting trailers, fertiliser spreaders and spray rigs. Such vehicles tend to spread large amounts of mud rapidly in the inter-rows, headlands and access roads.

Bananas need regular fungicide application to control leaf diseases. Conditions during the summer wet season are ideal for leaf diseases, and 20–22 sprays are required each year (Peterson 2001). The cooler, drier winter and spring months do not favour disease development and offer a significant 'window' to control and manage disease infection.

Figure 2.3 Motorised bagging machine in northern Queensland

Photograph: D Peasley

South-east Queensland and northern New South Wales

Climate

Both South-East Queensland and northern New South Wales have humid subtropical climates with a cooler and drier winter and spring (June–October). Rainfall (Figure 2.2) is predominantly (70%) during summer and early autumn (December–March), and tends to be less extreme than in north Queensland. Irrigation is used in some cases to supplement rainfall during drier periods. During the wet summer and early autumn, infection pressure for leaf diseases is high, requiring the application of 4–6 sprays to provide effective control (Peasley and Baker 2001).

Topography and soils

Production in South-East Queensland and northern New South Wales is limited to wind-protected frost-free slopes, with isolated plantations located on the frost-free plateau areas. North to North-East

facing slopes protected from southerly winds are considered the best locations for banana production (Peasley and Baker 2001). Although banana growing areas are mainly confined to slopes, production still occurs at a relatively low elevation, as evidenced by the elevation (below 20 m) of the key banana growing towns of Murwillumbah and Coffs Harbour.

Soils are generally podsolic clays or shales, with well-drained basaltic krasnozems on plateau areas and isolated slopes, and in pockets scattered throughout.

Aside from specially constructed roadways, mechanisation is limited by the gradient. This has the effect of reducing the movement of surface soil and hence the rate of transfer of soil-borne pests. Soilborne pests can, however, be transferred during periods of high rainfall with soil run.

Production and handling systems

Production is characterised by a large number of small plantings: approximately 1200 farms with an average size of three hectares (ABGC 2005). The holdings are largely family-operated, with size limited by topography. Climate limits year-round production, as well as the quality of Cavendish dessert bananas.

Carnarvon, Western Australia

Climate

Carnarvon is a semi-arid subtropical area with high summer temperatures and mild temperatures in winter and spring (Figure 2.2). Humidity is low all year. Conditions are not favourable for infection and spread of leaf diseases, and spraying for control is not required. Irrigation is essential year round as Carnarvon only receives an annual average rainfall of 226 mm.

Topography and soils

Carnarvon is located at an elevation of four metres and banana production is restricted to the sandy loam alluvial floodplain of the Gascoyne River. Under-canopy irrigation provides favourable conditions for infection and the spread of soilborne diseases and nematodes. The use of wheeled vehicles further increases the likelihood of spread of soilborne pests.

Production and handling systems

The banana production area covers about 132 hectares and is made up of 65 family-operated farms averaging two hectares each (ABGC 2005). Small holdings limit the economy of mechanisation, although the flat topography is suitable. Group marketing of fruit is increasing.

Kununurra, Western Australia

Climate

Kununurra has a semi-arid, monsoonal climate with wet and dry seasons. The area receives 82% of its annual rainfall between December and March (summer wet season), with 18% falling between April and November (Figure 2.2). The combination of high temperatures, high rainfall and high humidity favours disease and pest development, rapid growth, high plant respiration rates and high soil temperatures (Richards 2001).

High temperatures during November and December cause climatic stress and consequent fruit quality problems in bananas. Concentrated rainfall from November–March and the likelihood of high winds and humidity can cause significant localised damage to bananas (Richards 2001). Leaf disease control is needed during the wet season.

Topography and soils

Most production occurs on the sandy loams of the plains and higher land near sandstone ridges and 25% of the production area is on cracking clays along river banks, levies and plains (Richards 2001). Conditions are favourable for the spread of soilborne diseases during the hot or wet season and when irrigation is used.

Production and handling systems

The banana production area consists of ten family-operated farms averaging about 14 hectares each (ABGC 2005). Field operations are limited by hot weather and by storms and flooding in the wet season. Many farms cease operation during the wet season because of transport difficulties. Mechanisation is very limited.

Darwin, Northern Territory

Climate

Darwin has a semi-arid monsoon climate (Figure 2.2), with distinct wet (December–March) and dry (April–November) seasons. During the wet season, the rainfall is characterised by very high intensity falls of short duration, causing localised run-off even on well-drained and relatively level soils. The wet season is more intense than in Kununurra, and the dry season is humid. Temperatures during summer are more suitable for banana production than in Kununurra.

Topography and soils

Topography is flat to very slightly undulating. Banana production is on the tops and side slopes of very low plateaus, which are generally criss-crossed by drainage lines. Rainfall across the tops of plateaus is less than 2%, and across the side slopes is 2–3%. During the dry season, there is no surface movement of soil or water. During the wet season, when there are frequent short periods of very high intensity rainfall, there is significant lateral movement of water, soil and surface organic matter (G Walduck, Northern Territory Department of Business, Industry and Regional Development, pers comm 3 April 2002).

Production and handling systems

There are four banana farms in the Darwin region; averaging 50 hectares each (ABGC 2005). While mechanised operations are feasible, the area has been exposed to Panama disease (*Fusarium oxysporum* f. sp. *cubense* tropical race 4) and consequently mechanised operations have been limited to reduce the risk of spreading this disease.

Effects of rainfall, irrigation and temperature on pests and diseases in Australia

For several pests and diseases of bananas (black Sigatoka, freckle and Moko), the availability of water and the ambient temperature are important for their rate of development and the severity of disease. Consequently, the following information is relevant for the assessment of their risks.

Rainfall and irrigation

The average number of days per year on which more than 5 mm of rainfall is received varies from 50–75 in the banana grower areas of Australia (LGAs in which bananas are grown commercially), and from 30–50 in the main population centres in the other areas (Figure 2.4). Rainfall distribution also varies between these two areas, with mainly summer rainfall in the grower areas and winter rainfall in the other areas. The rainfall may be supplemented with irrigation in many commercial banana plantations and home garden situations and, to a lesser extent, in some other plant communities.

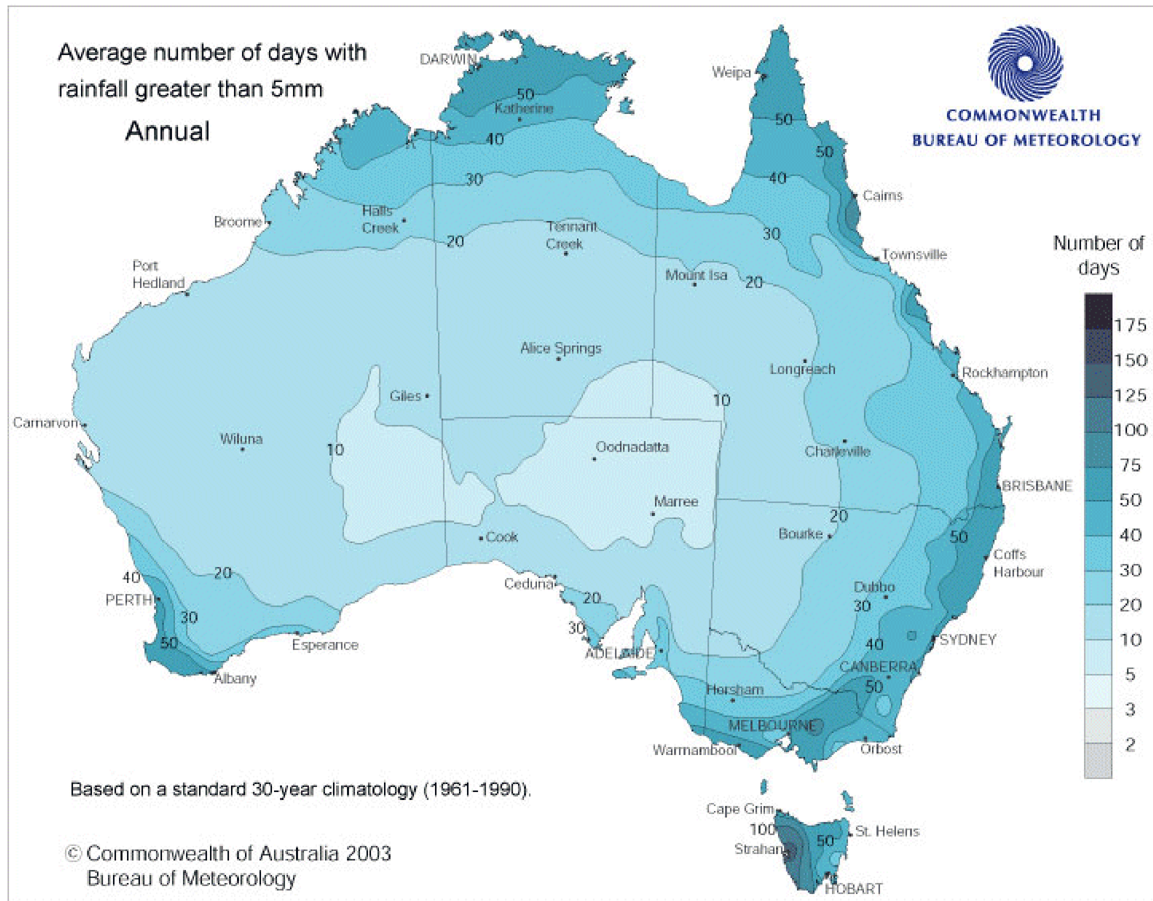
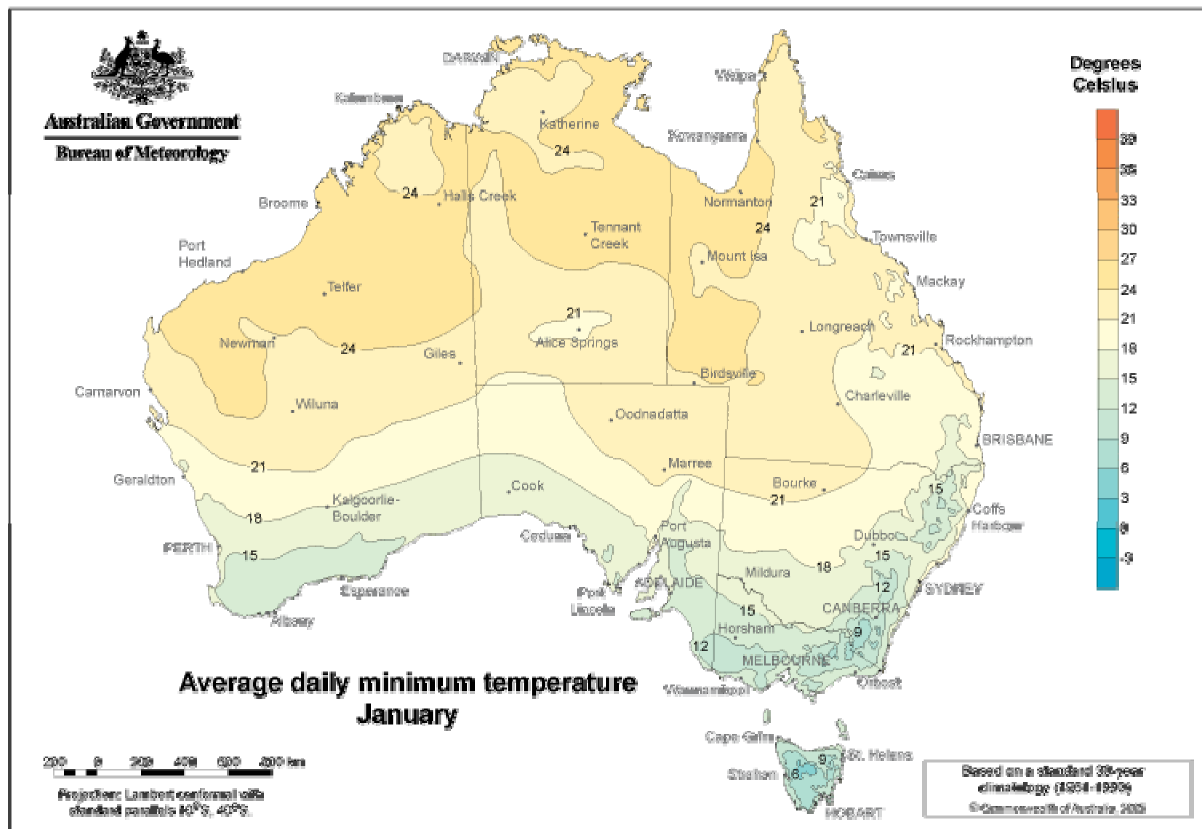


Figure 2.4 The average number of days per year with more than 5 mm of rain per day

Temperature

The average minimum and maximum daily temperatures in Australia for January and July are captured in Figure 2.5 and Figure 2.6.



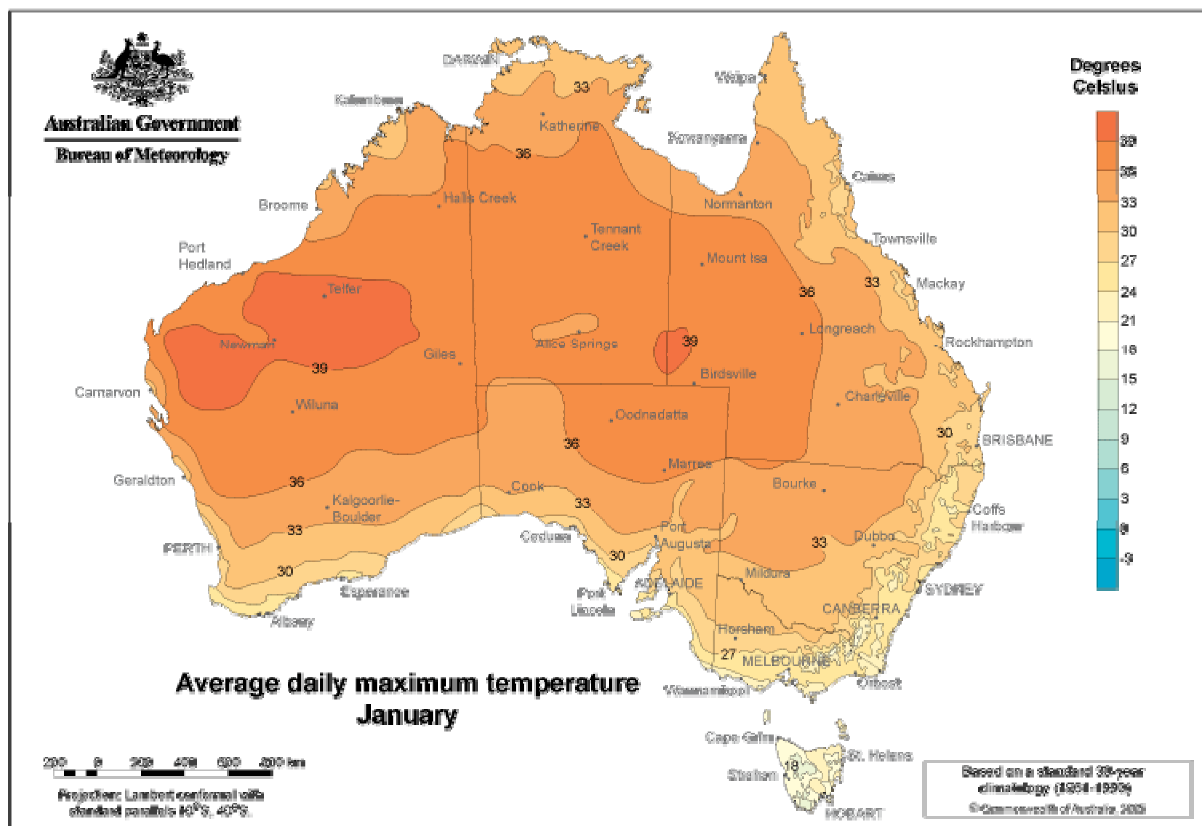


Figure 2.5 The average minimum and maximum monthly temperatures for January

Figure 2.6 The average minimum and maximum monthly temperatures for July

Appendix 3. Pathogen categorisation

Chapter 4 of Part B outlines the pest categorisation process. Pests that could potentially be associated with bananas in the Philippines were categorised according to their presence or absence in Australia. The descriptions include information on their regulatory status (where applicable), their potential for being present on the pathway (associated with banana fruit), their potential for establishment and spread in Australia, and the potential consequences of their establishment and spread.

This appendix lists the results of the categorisation of pathogens associated with banana plants and fruit in the Philippines. PRAs for the pests determined as needing further consideration using either quantitative or qualitative risk assessment methods are provided in chapters 9–13 of Part B.

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
BACTERIA							
Enterobacteriales: Enterobacteriaceae							
Pectobacterium carotovorum subsp. *carotovorum* (Jones 1901) Hauben et al 1999 emend. Gardan et al 2003 Syn. = *Erwinia carotovora* (Jones) Bergey, Harrison, Breed, Hammer and Huntoon 1923	Bacterial rhizome rot, corm rot	Yes BPI (2001); San Juan (1980); Persley and Cooke (1993)	Yes Qld Pegg et al (1974)				No
Burkholderiales: Ralstoniaceae							
Ralstonia solanacearum (Smith 1896) Yabuuchi et al 1996, comb. nov. (IJSB 1996) race 2 Syn. = *Pseudomonas solanacearum* (Smith 1896) Smith 1914 race 2	Moko bacterial wilt; Bugtok (= bacterial hard pulp)	Yes Jones (2000); BPI (2001)	No Hyde et al (1992); Jones (2000)	Yes Jones (2000); Soguilon (2003a)	Feasible	Significant. One of the most important diseases of bananas Jones (2000)	Yes
FUNGI							
ASCOMYCOTA							
ASCOMYCETES							
Botryosphaeriaceae							
Lasiodiplodia theobromae (Pat.), Griffon and Maubl. Syn. = *Botryodiplodia theobromae* Pat. [Teleomorph: *Botryosphaeria rhodina* (Berk. and MA Curtis) Arx]	Crown rot; finger rot; main stalk rot; peduncle rot	Yes Punithalingam (1976); Slabaugh (1994)	Yes Known from NSW, NT, Qld, SA, WA (APPD 2008)				No
Ceratocystidaceae							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Ceratocystis paradoxa (Dade) C. Moreau Syn. = *Ceratostomella paradoxa* Dade; *Ophiostoma paradoxum* (Dade) Nannf. Anamorph: *Chalara paradoxa* (De Seynes) Sacc. Syn. = *Thielaviopsis paradoxa* (De Seynes) Höhn.	Ceratocystis fruit rot; corm rot; crown rot; main stalk rot; stem-end rot	Yes CMI (1981)	Yes Known from NSW (bananas), QLD and WA (APPD 2008)				No
Glomerellaceae							
Colletotrichum musae (Berk. and MA Curtis) Arx (Anamorphic *Glomerella*)	Anthracnose; Central American fruit speckle (component); crown rot; fungal scald; main stalk rot; peduncle rot; stem-end rot	Yes Agati (1922); BPI (2000)	Yes Known from NSW, Qld, Vic, WA (APPD 2008); Hayden et al (1994)				No
Magnaporthaceae							
Pyricularia grisea (Cooke) Sacc. Teleomorph: *Magnaporthe grisea* (Hebert) Barr. Ascomata have not been reported from nature (Zeigler 1998)	Pitting	Yes Jones and Stover (2000); BPI (2001)	Yes Known from Poaceae and Zingiberaceae from NSW, Qld and WA but there are no records of *M. grisea* or *P. grisea* on *Musa* (APPD 2008). Molecular studies indicate that *P. grisea* exists as clonal populations (Shull and Hamer 1994; Zeigler 1998)				No
Mycosphaerellaceae							
Cercospora hayi alpouzos (Anamorphic *Mycosphaerella*) Crous and Braun (2003) accepted this species Syn. = *Cercospora apii* Fresen. emend Crous & U Braun	Brown spot; diamond spot (component)	Yes PCCARD (1988) This is a Central American fungus introduced to the Philippines	Yes APPD (2008)				No
Cladosporium musae EW Mason Syn. = *Periconiella sapienturnicola* Siboe	Cladosporium leaf speckle	Yes PCCARD (1988)	Yes Known from *Musa* in Qld (Pont 1960b), NT and WA (APPD 2008)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Guignardia musae Racib. Anamorph: *Phyllosticta musarum* (Cooke) van der Aa	Freckle	Yes Lee (1922); Jones (2000)	Yes Known from *Musa* in NSW, NT, Qld, WA (APPD 2008; Jones and Alcorn 1982). Exotic strains are of quarantine concern (Jones 2000; Condé 2001)	Yes On leaf and fruit (Jones 2000; Jones and Stover 2000)	Feasible	Significant. Though the fungus infects living tissues, it forms spore-producing structures only in necrotic tissues (Ploetz et al 2003). The destruction of leaf tissue may depress yield, but the major impact is blemishes of the peel, making the fruit unattractive to consumers.	Yes
Mycosphaerella fijiensis M. Morelet Syn. = *M. fijiensis* var. *difformis* JL Mulder and RH Stover Anamorph: *Pseudocercospora fijiensis* (M. Morelet) Deighton Syn. = *Paracercospora fijiensis* (M Morelet) Deighton; *Cercospora fijiensis* M Morelet)	Black leaf streak = black Sigatoka leaf spot; mixed ripe fruit; premature ripening	Yes Johnston (1965); BPI (2001)	Yes Known in Qld (APPD 2008) (restricted and under official control); (Hayden et al 2003a)	Yes In trash (Pasberg Gauhl et al 2000)	Feasible	Significant. Black Sigatoka is a leaf spot disease which infects and causes disease primarily on the leaf lamina and to a lesser extent on the mid-rib tissue. It has not been reported to infect the flowers, flower bracts, pseudostem, corm, roots or fruit of Cavendish bananas (Carlier et al 2000)	Yes
Mycosphaerella musicola R Leach ex JL Mulder Anamorph: *Pseudocercospora musae* (Zimm.) Deighton Syn. = *Cercospora musae* Zimm	Mixed ripe fruit; premature ripening; Sigatoka leaf spot = yellow Sigatoka leaf spot	Yes Stover (1962); Jones (2000)	Yes Known in NSW, NT, Qld and WA (APPD 2008); Hayden et al (2005)				No
Nectriaceae							
Fusarium moniliforme Sheldon The name *F. moniliforme* has been rejected because it has been applied in the taxonomies of Booth (1971) and Nelson et al (1983) to a multitude of phylogenetically distinct species that are now placed in the *Gibberella fujikuroi* clade (O'Donnell et al 1998, 2000). *Fusarium sacchari* also occurs. In the strict sense *F. moniliforme* is a later synonym of *F. verticillioides* (Nirenberg 1976). *Fusarium verticillioides* is known to infect banana fruit (Mirete et al 2004; Moretti et al 2004). Nirenberg and O'Donnell (1998) described *Fusarium concentricum* from *Musa*	Crown rot; black heart; pseudostem heart rot; root and rhizome rot – associated with nematode lesions	Yes Cook (1981) No records found from the Philippines from bananas of members of the *Gibberella fujikuroi* complex including: *F. verticillioides* *F. sacchari* or *F. concentricum*	Yes APPD (2008). There are no records on APPD (2008) of *F. verticillioides* *F. sacchari* or *F. concentricum* Mycotoxin production by these fusaria is of concern (Mirete et al 2004; Moretti et al 2004)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Fusarium oxysporum Schltdl. f. sp. *cubense* (EF Sm.) WC Snyder and HN Hansen (race 4) (Anamorphic *Gibberella*)	Panama disease, fusarium wilt	Yes Bentley et al (1998); BPI (2001)	Yes NSW, NT, and Qld (Pegg et al 1994; APPD 2008); Under quarantine control in S. Qld, N. NSW and NT				No
Fusarium pallidoroseum (Cooke) Sacc. (Anamorphic *Gibberella*)	Crown rot; diamond fruit spot (component); fungal scald; peduncle rot	Yes PCCARD (1988)	Yes Jones and Stover (2000); NT and QLD (APPD 2008)				No
Fusarium roseum Link (Anamorph of *Gibberella zeae* (Schwein.) Petch *Gibberella zeae* comprises seven or more phylogenetic lineages (Ward et al 2002) some of which are interfertile (Bowden and Leslie 1999). Some authors consider *F. roseum* a *nomen dubium*; O'Donnell et al (2004) determined there were nine species in the *Fusarium graminearum* clade	Crown rot; fruit rot	Yes PCCARD (1988)	Yes Many records from Australia (APPD 2008) but there are no records of either *F. roseum* or *G. zeae* on *Musa*			No Recorded as a secondary invader of injured tissue (Stover 1972)	No
Pleosporaceae							
Curvularia lunata (Wakker) Boedjin Teleomorph: *Cochliobolus lunatus* RR Nelson and Haasis	Black leaf spot	Yes Lee et al (1986)	Yes APPD (2008) lists *C. lunatus* from NT, Qld, Vic, and on *Musa* in Qld, and *Heliconia* in NT				No
Trichocomaceae							
Aspergillus niger Tiegh. (Anamorphic Trichocomaceae)	Collar rot; fruit rot	Yes Morantte et al (1986)	Yes very widely distributed in Australia (APPD 2008)				No
Xylariaceae							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Rosellinia bunodes (Berk. and Broome) Sacc. Syn. = *Rosellinia echinate* Berk. and Broome; *Rosellinia zingiberi* Berk. and Broome Molecular studies have shown that most of the older morphological species of *Rosellinia* are species complexes (Bahl et al 2005)	Black root rot	Yes CAB International (2006)	No	No On crown (Jones 2000). Many species of Xylariaceae are endophytes of living foliage (Petrini and Petrini 2005). Xylariaceous species were common endophytes of leaves of wild *Musa acuminata* in Thailand (Photita et al 2001)	Feasible	Minor. AAA cultivars more resistant than plantains (Ploetz and Pegg 2000)	No
Anamorphic Ascomycetes							
Cordana musae (Zimm.) Höhn	Cordana leaf spot	Yes BPI (2001)	Yes Simmonds (1966); Jones (2000); APPD (2008)				No
Deightoniella torulosa (Syd.) Ellis	Black-tip; Central American fruit speckle (component); cigar-end rot; damping-off; Deightoniella fruit speckle; Deightoniella leaf spot	Yes BPI (2001)	Yes Known from *Musa* in NSW, Qld and WA (APPD 2008); Persley and Cooke (1993); Shivas (1989); Simmonds (1966)				No
Ramichloridium musae (Stahel ex EBEllis) De Hoog Syn. = *Periconiella musae* Stahel ex MB Ellis; *Veronaea musae* Stahel ex MB Ellis; *Chloridium musae* Stahel (Anamorphic Herpotrichellaceae)	Ramichloridium leaf speckle	Yes PCCARD (1988)	Yes Known from *Musa* from Qld (Pont 1960b; APPD 2008)				No
BASIDIOMYCOTA							
BASIDIOMYCETES							
Agaricomycetidae							
Marasmiellus inoderma (Berk.) Singer = *Marasmius semiustus* Berk. and Curtis	Marasmiellus pseudostem and root rot	Yes BPI (2001)	Yes Known from *Musa* from NSW and Qld (APPD 2008)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
UREDINIO-MYCETES							
Pucciniaceae							
Uredo musae Cummins	Rust	Yes CIE (1971)	Yes Qld (APPD 2008); Christmas Island (Shivas 1989)				No
Uredo musicola Yen	Rust	Yes Yen (1974)	No (APPD 2008)	Yes In leaf trash (Yen 1974)	Feasible	No information on economic importance. Possibly a threat to indigenous taxa of *Musa*	No
Uromyces musae Henn.	Rust	Yes PCCARD (1988); Teodoro (1937)	No (APPD 2008)	Yes In leaf trash (Jones 2000)	Feasible	No information on economic importance. Possibly a threat to indigenous taxa of *Musa*	No
NEMATODES							
NEMATODA Tylenchida							
Heteroderidae							
Rotylenchus reniformis (Linford and Oliveira 1940)	Reniform nematode	Yes Villanueva (2003)	Yes WA (McLeod et al 1994)				No
Hoploplaimidae							
Helicotylenchus multicinctus (Cobb 1893)	Banana spiral nematode	Yes CAB International 2006	Yes NSW, NT, Qld, SA, WA (McLeod et al 1994)				No
Hoplolaimus seinhorsti Luc	Lance nematode	Yes CAB International 2006	Yes Qld, NT (McLeod et al 1994)				No
Meloidogynidae							
Meloidogyne arenaria (Neal 1859)	Root knot nematode	Yes Taylor et al (1982)	Yes Qld, WA (Taylor et al 1982; McLeod et al 1994)				No
Meloidogyne incognita (Kofoid and White 1919)	Root knot nematode	Yes Villanueva (2003)	Yes NSW, NT, Qld (Simmonds 1966; McLeod et al 1994)				No
Pratylenchidae							
Radopholus similis (Cobb 1893) Thorne	Burrowing nematode	Yes Booth and Stover (1974)	Yes NSW, NT, Qld, WA (Persley and Cooke 1993; McLeod et al 1994;)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
VIRUSES							
Bromoviridae							
Cucumber mosaic virus	Mosaic	Yes BPI (2001)	Yes NSW, Qld (Persley and Cooke 1993; Jones 2000)				No
Caulimoviridae							
Banana streak virus	Streak	Yes Jones (2000)	Yes Qld (Persley and Cooke 1993; Jones 2000)				No
Nanoviridae							
Banana bunchy top virus	Bunchy top	Yes Jones (2000)	Yes NSW, S.E. Qld (under official control) (Jones 2000)	Yes Othman et al (1996)	Feasible	The normal phloem tissue of infected banana plants is replaced, wholly or in part, by a morbid tissue in which areas of obliteration or necrosis would occur (Magee 1939)	Yes
Abacá bunchy top virus This virus is currently undergoing taxonomic separation from BBTV based on molecular and serological detection assays (J Thomas, pers comm 25 July 2006)		Unknown	No (J Thomas, pers comm 25 July 2006)	Yes	Feasible	Biologically similar to BBTV (J Thomas, pers comm 25 July 2006)	Yes
Potyviridae							
Abacá mosaic virus	Abacá mosaic, sugarcane mosaic	Yes Jones (2000)	No Jones (2000)	No This virus is not found in commercial Cavendish plantations in the Philippines (BPI 2002a)			No
Banana bract mosaic virus	Bract mosaic	Yes Jones (2000)	No Jones (2000)	Yes Rodoni et al (1997)	Feasible	BBrMV results in mosaic patterns in affected tissues. Suckering can be suppressed and suckers that do emerge can be distorted and deeply pigmented (Thomas et al 2000)	Yes

Appendix 4. Arthropod categorisation

Chapter 4 of Part B outlines the pest categorisation process. Pests that could potentially be associated with bananas in the Philippines were categorised according to their presence or absence in Australia. The descriptions include information on their regulatory status (where applicable), their potential for being present on the pathway (associated with banana fruit), their potential for establishment and spread in Australia, and the potential consequences of their establishment and spread.

This appendix lists the results of the categorisation of arthropods associated with banana plants and fruit in the Philippines. PRAs for the pests determined as needing further consideration using qualitative risk assessment methods are provided in chapters 14–19 of Part B. Only pests of above ground plant parts are listed; for a comprehensive listing of pests of *Musa spp*. refer to the previous revised draft IRA report on the importation of fresh bananas from the Philippines, February 2004 (BA 2004).

Note: Current Biosecurity Australia and AQIS policy for the interception of pests not known to be pests of the imported commodity (contaminants) requires that the consignment is held until the quarantine status of the contaminants is determined. If the pests are determined to be of a quarantinable status, the importer is given the options of treatment of the consignment to remove the pest, re-export or destruction of the consignment.

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
ARTHROPODA: INSECTA							
Coleoptera (beetles)							
Anthribidae (Fungus weevils)							
Araecerus coffeae (Fabricius 1801) Misidentified as *Araecerus fasciculatus* (De Geer 1775) (see Zimmerman 1994)	Coffee bean weevil	Yes Waterhouse (1993); Mphuru (1974)	Yes WA, Qld, NSW (APPD 2005; Zimmerman 1994; Zeck 1943)	No Larvae normally feed in seeds, especially coffee beans, but also dead, fungus-infested tissues of many plants			No
Curculionidae (Weevils)							
Cosmopolites sordidus (Germar 1824)	Banana weevil borer; banana root weevil	Yes PCARRD (1988)	Yes*[1] Qld, NSW (Pinese and Piper 1994; Zimmerman 1993)	No Larvae feed and develop entirely within the corm of banana below the soil surface (Pinese and Piper 1994; Zimmerman 1993)			No

[1] 'Yes*' indicates that this species is present but not widely distributed and being officially controlled, or where regional freedoms exist within Australia, based on administered interstate measures.

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Odoiporus longicollis (Olivier 1807)	Banana stem weevil; banana stem borer	Yes Uichanco (1936)	No Robbs et al (1995)	No Larvae feed on the pseudostem of banana above ground (Pinese and Piper 1994; Kalshoven and van der Laan 1981; Isahaque 1978; Dutt and Maiti 1972)			No
Philicoptus demissus (Heller 1929)	Peel-scarring weevil	Yes Stephens (1984)	No Stephens (1984)	Yes Adults feed on base of leaf lamina, leaf petioles, ridges of leaf veins, flower bracts and ridges of young banana fingers (BPI 2001; Stephens 1984)	Feasible	Significant Causes scarring on banana peel, rendering fruit unfit for export (BPI 2001)	Yes
Philicoptus iliganus (Heller 1929)	Peel-scarring weevil	Yes PCARRD (1988); Stephens (1984)	No Stephens (1984)	Yes Adults mostly feed on the peel of young banana fingers (BPI 2001; Stephens 1984)	Feasible	Significant Adults are polyphagous and cause scarring on banana peel, rendering fruit unfit for export (BPI 2001)	Yes
Philicoptus strigifrons (Heller 1929)	Peel-scarring weevil	Yes Stephens (1984)	No Stephens (1984)	Yes Adults feed on flower bracts and peel of young banana fingers (Stephens 1984)	Feasible	Significant Causes scarring on banana peel, rendering fruit unfit for export (BPI 2001)	Yes
Philicoptus sp.1	Peel-scarring weevil	Yes Stephens (1984)	Uncertain	Yes Adults feed on flower bracts and peel of young banana fingers (Stephens 1984)	Feasible	Significant Causes scarring on banana peel, rendering fruit unfit for export (BPI 2001)	Yes
Philicoptus sp.2	Peel-scarring weevil	Yes Stephens (1984)	Uncertain	Yes Adults feed on flower bracts and peel of young banana fingers (Stephens 1984)	Feasible	Significant Causes scarring on banana peel, rendering fruit unfit for export (BPI 2001)	Yes
Scarabaeidae (Scarabs)							
Oryctes rhinoceros (Linnaeus 1758)	Rhinoceros beetle	Yes Waterhouse (1993)	No Mohan (2005)	No Eggs laid in and larvae feed in rotting vegetative matter (Bedford 1980). A single historical record from Australia, but identification not verified.			No
Diptera (flies)							
Muscidae (Muscids, house flies, stable flies)							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Atherigona orientalis Schiner 1868	Pepper fruit fly, tomato fly.	Yes Pont (1992)	Yes WA, NT, Qld, NSW (Cahill 1992)				No
Stratiomyidae (Soldier flies)							
Hermetia illucens (Linnaeus 1758)	American soldier fly	Yes Rueda et al (1990)	Yes* Qld (McCallan 1974; Warburton and Hallman 2002) WA (APPD 2008)	Yes Eggs are laid between fingers but larvae do not penetrate green bananas (Stephens 1975). Larvae typically feed on rotting vegetation (Newby R 1997)			No
Tephritidae (Fruit flies)							
Bactrocera occipitalis (Bezzi 1919)	Fruit fly	Yes Drew and Hancock (1994)	No Drew (1988)	Yes Eggs must be deposited in ripening or ripe fruit and larvae feed on rotting banana flesh (Brown 1998)	Feasible	Significant Both *B. occipitalis* and *B. philippinensis* cause 10–30% damage to bananas on Palau (Sengebau et al 2005; Republic of Palau 2002)	Yes
Bactrocera philippinensis Drew and Hancock 1994	Philippines fruit fly	Yes Drew and Hancock (1994)	No Drew and Hancock (1994)	Yes Eggs must be deposited in ripening or ripe fruit and larvae feed on rotting banana flesh (Brown 1998)	Feasible	Significant Both *B. occipitalis* and *B. philippinensis* cause 10–30% damage to bananas on Palau (Sengebau et al 2005; Republic of Palau 2002)	Yes
Hemiptera (true bugs including aphids, lace bugs, mealybugs, scales, whiteflies)							
Aleyrodidae (Whiteflies)							
Aleurocanthus woglumi (Ashby 1915)	Citrus white fly	Yes Martin (1999); Mound and Halsey (1978)	No Martin (1999); Mound and Halsey (1978)	No Eggs laid on and immature stages form dense colonies on underside of leaves (Enkerlin 1976; Shaw 1950)			No
Aleurodicus dispersus (Russell 1965)	Spiralling white fly	Yes Lit and Calilung (1994); Waterhouse (1993)	Yes* NT, Qld (APPD 2005; Carver and Reid 1996)	No Normally deposits eggs on underside of foliage, very occasionally on fruit (Lambkin 2004), however fruit not considered to provide a viable pathway (DAFWA 2007			No
Aphididae (Aphids)							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Aphis gossypii (Glover 1877)	Cotton aphid; melon aphid	Yes Waterhouse (1993)	Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Carver and Reid 1996)				No
Pentalonia nigronervosa (Coquerel 1859)	Banana aphid	Yes Lomerio and Calilung (1993); PCARRD (1988)	Yes WA, NT, Qld, NSW (APPD 2005; Pinese and Piper 1994)	Yes Most frequently found near the base of suckers; occasionally found on fruit-bearing plants (Robson et al 2006).	No Would be unable to survive shipment period of 10 - 14 days		No Refer to the BBTV PRA
Rhopalosiphum maidis (Fitch 1856) Syn. = *Aphis maidis* (Fitch 1856) [Aphididae]	Corn aphid; maize aphid	Yes Waterhouse (1993)	Yes WA, NT, Qld, NSW, Vic (APPD 2005)				No
Coccidae (Soft scales, wax scales)							
Ceroplastes rubens Maskell 1893	Pink wax scale	Yes Ben-Dov *et al*. (2008a)	Yes Qin & Gullan (1994)				No
Coccus hesperidum Linnaeus 1758	Brown soft scale	Yes Sugimoto (1994); Swirski et al (1997)	Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Ben-Dov 2006a)				No
Coccus longulus (Douglas 1887)	Long brown scale	Yes Ben-Dov *et al*. (2008b)	Yes Ben-Dov *et al*. (2008b)				No
Saissetia coffeae (Walker, 1852)	Hemispherical scale	Yes Ben-Dov *et al*. (2008c)	Yes Ben-Dov *et al*. (2008c)				No
Vinsonia stellifera (Westwood 1871)Coccus	Stellate scale	Yes Ben-Dov *et al*. (2008d)Yes Sugimoto (1994); Swirski et al (1997)	Yes NT Qin & Gullan (1994)Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Ben-Dov 1993)	No Feeds on foliage (Blackwood and Pratt 2007)			No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Diaspididae (Armoured scales, hard scales)							
Abgrallaspis cyanophylli (Signoret 1869)	Cyanophyllum scale	Yes Sugimoto (1994); Velasquez (1971)	Yes* NT, Qld, NSW, Tas (APPD 2005; CSIRO-AFFA 2004a)	Yes Intercepted on bananas to Japan (Sugimoto 1994)	Feasible	Significant Damages *Psidium guajava* in Fiji (Lever 1945) and tea in Papua New Guinea (Greve and Ismay 1983). It is often a serious pest of palms and attacks several fruits including custard apple, avocado, banana, guava, macadamia and mango (Kessing and Mau 2007)	Yes (WA only)
Aonidiella aurantii (Maskell 1878)	Californian red scale	Yes Velasquez (1971)	Yes WA, NT, Qld, NSW, Vic (APPD 2005); NSW (Laing 1929; Rosen and DeBach 1978); Qld (Brimblecombe 1962); Vic (Laing 1929)				No
Aonidiella orientalis (Newstead 1894)	Oriental scale	Yes Velasquez (1971)	Yes WA, NT, Qld (APPD 2005)				No
Aspidiotus coryphae Cockerell and Robinson 1915	Corypha scale	Yes Sugimoto (1994); Velasquez (1971)	No (APPD 2008)	Yes Intercepted on bananas to Japan (Sugimoto 1994)	Feasible	Significant Other hosts are cycads and palms (Ben-Dov et al 2005a)	Yes
Aspidiotus destructor Signoret 1869	Transparent scale; coconut scale	Yes Sugimoto (1994); Waterhouse (1993); Velasquez (1971)	Yes WA, NT, Qld, NSW (APPD 2005)				No
Aspidiotus excisus Green 1896	Cyanotis scale	Yes Sugimoto (1994); Velasquez (1971)	No (APPD 2008)	Yes Intercepted on bananas to Japan (Sugimoto 1994)	Feasible	Significant A polyphagous species considered to be a pest of ornamental plants (Davidson and Miller 1990)	Yes
Chrysomphalus aonidum (Linnaeus 1758) Syn. = *Chrysomphalus ficus* (Ashmead 1880)	Circular black scale; Florida red scale	Yes Velasquez (1971)	Yes WA, NT, Qld, NSW (APPD 2005)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Chrysomphalus dictyospermi (Morgan 1889)	Spanish red scale; Dictyospermum scale	Yes Velasquez (1971)	Yes WA, NT, Qld, NSW (APPD 2005)				No
Hemiberlesia lataniae (Signoret 1869)	Latania scale; palm scale	Yes Sugimoto (1994); Velasquez (1971); Green (1937)	Yes WA, NT, Qld, NSW (APPD 2005; Waite 1988)				No
Hemiberlesia palmae (Cockerell 1892) Syn. = *Aspidiotus palmae* Cockerell; *Abgrallaspis palmae* (Cockerell); *Abrallaspis palmae* (Cockerell)	Citrus black scale; Tropical palm scale	Yes PCARRD (1988); Sugimoto (1994); Velasquez (1971)	Yes* Qld (APPD 2008)	Yes Intercepted on bananas to Japan (Sugimoto 1994); colonises and sucks sap of banana leaf (PCARRD 1988)	Feasible	Significant A polyphagous pest of banana, coconut palm, manihot, oil palm and cocoa (Chua and Wood, 1990) in the tropics, of tea in Malaysia (Watson 2005) and known to damage orchids in greenhouses (Ben-Dov et al 2006b)	Yes (WA only)
Hemiberlesia rapax (Comstock 1881)	Greedy scale, apacious scale	Yes Sugimoto (1994); Nakahara (1982)	Yes WA, Qld, NSW, Vic, Tas (APPD 2005; Brimblecombe 1968; Froggatt 1914)				No
Hemiberlesia lataniae (Signoret 1869)	Latania scale; palm scale	Yes Sugimoto (1994); Velasquez (1971); Green (1937)	Yes WA, NT, Qld, NSW (APPD 2008; Waite 1988)				No
Lepidosaphes gloverii Tau 1978	Citrus long scale	Yes Ben-Dov *et al*. (2008e)	Yes Ben-Dov *et al*. (2008e)				No
Pinnaspis aspidistrae aspidistrae (Signoret 1869)	Aspidistra scale	Yes Ben-Dov *et al*. (2008f)	Yes Ben-Dov *et al*. (2008f)				No
Pinnaspis musae Takagi 1963	Armoured scale, hard scale	Yes Takagi (1963); Sugimoto (1994)	No (APPD 2008)	Yes Intercepted on bananas to Japan (Takagi 1963; Sugimoto 1994)	Feasible	Significant	Yes
Pinnaspis strachani (Cooley 1899)	Lesser snow scale, hibiscus snow scale, cotton white scale	Yes (Nakahara 1982; Ferris 1947)	Yes WA, NT, Qld, NSW (APPD 2005; Brookes 1964)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Pseudaulacaspis cockerelli (Cooley 1897)	Mango white scale, false oleander scale	Yes Sugimoto (1994); Nakahara (1982)	Yes* NT, Qld, NSW (APPD 2005; Green 1914)	Yes Intercepted on bananas to Japan (Sugimoto 1994)	Feasible	Significant Considered the most serious pest of ornamental plants in Florida (Hamon and Fasulo 2000) and is also an important pest of mango (Crane and Campbell 1994)	Yes (WA only)
Selenaspidus articulatus (Morgan 1889)	Rufous scale	Yes Ben-Dov *et al.* (2008g)	Yes But absent from WA Ben-Dov *et al.* (2008g)	Yes Leaves and sometimes fruit/pods (Watson 2008)	Feasible	Yes Important pest of many commercial plant species (Watson 2008)	Yes (WA only)
Margarodidae (Ground pearls, cottony cushion scales)							
Icerya aegyptiaca Douglas 1890	Breadfruit mealybug	Yes Ben-Dov et al (2005a)	Yes WA, NT, Qld (APPD 2005)				No
Icerya seychellarum seychellarum (Westwood 1855) [The other subspecies, *Icerya seychellarum cristata* Newstead 1908 is restricted to Madagascar]	Seychelles scale	Yes Sugimoto (1994)	Yes WA, NT, Qld (APPD 2005)				No
Pseudococcidae (Mealybugs)							
Dysmicoccus brevipes (Cockerell 1893)	Pineapple mealybug	Yes Williams (2004); Sugimoto (1994); Waterhouse (1993)	Yes WA, NT, Qld, NSW (APPD 2005; Ben-Dov et al 2005a; Williams 1985)				No
Dysmicoccus neobrevipes Beardsley 1959	Grey pineapple mealybug, annona mealybug	Yes Ben-Dov et al (2005a); Williams (2004); PCARRD (1988); Sugimoto (1994)	No Ben-Dov et al (2005a)	Yes Intercepted on bananas to Japan (Sugimoto 1994; Ben-Dov et al 2005a)	Feasible	Significant A polyphagous species reported as being a pest of pineapple in Hawaii and of *Albizia saman* an important amenity tree in Thailand and Australia (Williams 2004; Williams and Watson 1988)	Yes

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Ferrisia virgata (Cockerell 1893)	Striped mealybug, grey mealybug, guava mealybug	Yes Williams (2004); Sugimoto (1994); Waterhouse (1993)	Yes WA, NT, Qld (APPD 2005; Williams 1985)				No
Maconellicoccus hirsutus (Green 1908)	Pink hibiscus mealybug	Yes Williams (2004)	Yes WA, NT, SA, Qld, NSW, Vic (APPD 2005; Ben-Dov et al 2005a)				No
Nipaecoccus nipae (Maskell 1893)	Spiked mealybug, buff coconut mealybug, nipa mealybug, avocado mealybug, sugarapple mealybug	Yes Lit et al (2006)	No Ben-Dov et al (2008h) record from an unspecified location. There is a single specimen record from an unspecified location (APPD 2008). The information claiming this species is present in Australia is very questionable specifically in relation to the information for the single record regarding location, timing and situation where the specimen was collected. Therefore the IRA team considered this record to be unreliable.	Yes CAB International (2006)	Feasible	Significant Polyphagous pest species affecting 80 genera in 43 plant families including avocado, banana, custard apple, guava, mango, grapes, olives, palms, and orchids. Damage caused by *N. nipae* may result in ornamental plants, fruit or cut flowers losing their market value (CAB International 2006).	Yes
Planococcus citri (Risso 1813)	Citrus mealybug	Yes Williams (2004); Sugimoto (1994); Waterhouse (1993)	Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Ben-Dov et al 2005a; Williams 1985)				No
Planococcus minor (Maskell 1897) Syn. = *Planococcus pacificus* (Cox 1981)	Passionvine mealybug, Pacific mealybug	Yes Ben-Dov et al (2005a); Williams (2004); Sugimoto (1994)	Yes* NT, SA, Qld, NSW (APPD 2005; Ben-Dov et al 2005a)	Yes Intercepted on bananas to Japan (Sugimoto 1994)	Feasible	Significant A minor pest of cacao and custard apple in Burma and one of the main pests of passionfruit in south-eastern Queensland (Williams 2004; Williams 1985)	Yes (WA only)

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Pseudococcus jackbeardsleyi Gimpel and Miller 1996 Previously misidentified as *P. elisae* Borchsenius, 1947; see Gimpel and Miller (1996)	Jack Beardsley mealybug	Yes Ben-Dov et al (2005a); Williams (2004); Lit and Calilung (1994)	No Ben-Dov et al (2005a)	Yes Intercepted on bananas to Japan (Sugimoto 1994) under the name *P. elisae*	Feasible	Significant A pest of banana, tomato, potato, pepper and *Hibiscus* (Gimpel and Miller 1996)	Yes
Pseudococcus longispinus (Targioni Tozzetti 1867)	Longtail mealybug	Yes Williams (2004); Lit and Calilung (1994); Sugimoto (1994)	Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Ben-Dov et al 2005a; Williams 1985)				No
Rastrococcus invadens Williams 1986	Mango mealybug	Yes Ben-Dov et al (2005a); Williams (2004)	No Ben-Dov et al (2005a)	No Observed on banana plants but not associated with the fruit (Ben-Dov et al 1994)			No
Rastrococcus spinosus (Robinson 1918)	Mealybug	Yes Ben-Dov et al (2005a); Williams (2004); Williams (1989)	No Ben-Dov et al (2005a); Williams (1989)				No
Tingidae (Lace bugs)							
Stephanitis typica (Distant 1903)	Banana lace bug, coconut lace bug	Yes Hoffmann (1935)	No Cassis and Gross (1995)	No Feeds on undersurface of banana leaf lamina (Tigvattnanont 1990; Cheng 1967; Hoffmann 1935)			No
Lepidoptera (butterflies, moths)							
Hesperiidae (Skippers)							
Erionota thrax (Linnaeus 1767) This species has been confused in the literature with *Pelopidas thrax* (Hübner 1821), which is a grass feeder that occurs in the Middle East with subspecies in Africa (CABI 2005).	Banana leaf roller, banana skipper	Yes De Jong and Treadaway (1993); Evans (1949)	No Braby (2000); Sands and Sands (1991)	No Feeds on leaves (BPI 2001; Ashari and Eveleens 1974)			No
Limacodidae (Cup moths, slug moths)							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Darna diducta (Snellen 1900) Syn. = *Ploneta diducta* (Snellen 1900)	Oil palm caterpillar	Yes Waterhouse (1993); Holloway et al (1987)	No Nielsen et al (1996)	No Feeds on leaves or leaflets (Holloway et al 1987)			No
Parasa lepida Cramer 1779	Nettle caterpillar	Yes Waterhouse (1993)		No Larvae feed on the leaves of a variety of plant species (Wakamura et al 2007)			No
Thosea philippina Holloway 1987		Yes Holloway et al (1987)	No Nielsen et al (1996)	No Feeds on leaves (Holloway et al 1987; Kalshoven and van der Laan 1981)			No
Thosea sinensis (Walker 1855) According to Holloway et al (1987) all references to *T. sinensis* should probably now refer to *T. philippina*.	Cup moth; assam nettle; saddle-back nettle	Yes Waterhouse (1993) This species is not present in the Philippines (Holloway et al 1987)					No
Lymantridae (Tussock moths)							
Olene mendosa (Hübner 1823)	Brown tussock moth	Yes Waterhouse (1993); Palomar (1998)	Yes Qld, NSW & NT(APPD 2008; Common 1990)	No Foliage feeder (Kuroko and Lewvanich 1993)			No
Noctuidae (Army worms, owlet moths)							
Spodoptera exigua (Hübner 1808)	Beet armyworm	Yes Ulrichs and Mewis (2004)	Yes WA, NT, SA, Qld, NSW, Vic (Nielsen et al 1996)				No
Spodoptera litura Fabricius 1775	Cluster caterpillar	Yes Waterhouse (1993)	Yes (APPD 2008)				No
Tiracola plagiata (Walker 1857)	Banana fruit caterpillar, cacao armyworm, plague caterpillar	Yes CAB International (2006)	Yes* Qld, NSW (APPD 2005; Nielsen et al 1996; Froggatt 1928)	Yes Larvae feed on foliage and fruit, with large larvae feeding deep into the fruit (Pinese and Piper 1994; Common 1990)	Feasible	Not significant This species causes sporadic damage that is more prevalent in hot summers after the wet season. It is rarely sufficient to merit specific treatment in Queensland banana plantations (Pinese and Piper 1994)	No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Nymphalidae (Brushfooted butterflies)							
Melanitis leda ismene (Cramer 1775) [Nymphalidae]	Green horned caterpillar, rice butterfly	Yes Dale (1994)	Yes* Qld (Braby 1995)	No Feeds on leaves (Braby 1995)			No
Psychidae (Case moths)							
Amatissa fuscescens (Snellen 1879) Syn. = *Cryptothelea fuscescens* (Snellen), see Norman et al (1995)	Bag worm	Yes PCARRD (1988); Kalshoven and van der Laan (1981)	No Nielsen et al (1996)	No Feeds on young shoots and leaves of banana (BPI 2001; Kalshoven and van der Laan 1981).			No
Pyralidae (Grass moths, snout moths)							
Cnaphalocrocis medinalis (Guenée 1854)	Rice leaf folder	Yes Barrion et al (1991)	Yes WA, NT, Qld, NSW (APPD 2005; Nielsen et al 1996)				No
Tirathaba rufivena (Walker 1864)	Coconut spiked moth, oil palm bunch moth	Yes CAB International (2006)	Yes* NT, Qld (APPD 2005; Nielsen et al 1996; Froggatt 1928)	Yes Froggatt (1928)	Feasible	Not significant This species has very little, if any, effect on coconut production in Malaysia and Tonga, and only causes short-term yield reductions in young coconut trees in Philippines (Waterhouse and Norris 1987). It is not recorded as being a major or minor pest in Queensland banana plant-ations (Pinese and Piper 1994)	No
Zygaenidae (Leaf skeletoniser moths)							
Artona catoxantha (Hampson 1892) Syn. = *Brachartona catoxantha* (Hampson 1892)	Coconut leaf moth	Yes van der Vecht (1950); Merino (1938)	No Nielsen et al (1996)	No Feeds on leaves (van der Vecht 1950)			No
Orthoptera: (Grasshoppers and crickets)							
Acrididae (Grasshoppers)							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Chondracris rosea (de Geer 1773)	Citrus locust, cotton locust	Yes Dammerman (1929)	No Mungai (1992)	No Feeds on leaves (Sun et al 2006)			No
Locusta migratoria Linnaeus 1758	Migratory locust	Yes Roffey (1972)	Yes WA, NT, Qld, NSW (Miller et al 2002; Farrow 1979)				No
Valanga nigricornis (Burmeister 1838)	Short horned grasshopper	Yes Waterhouse (1993)	Yes* Qld (Pinese and Piper 1994)	No Foliage feeder (Nair 2001) (Feeds on the leaves of banana, in extreme cases only leaving the midrib Gabriel 1975)			No
Thysanoptera (Thrips)							
Thripidae							
Chaetanaphothrips signipennis (Bagnall 1913)	Banana rust thrips; red rust thrips	Yes Reyes (1994); PCARRD (1988)	Yes* NT, Qld, NSW (APPD 2005; Williams et al 1990; Pitkin 1977; Froggatt 1928)	Yes Feeding on fruit causes 'rusting' and eggs are inserted below the surface of fruit (Pinese and Piper 1994; Williams et al 1990; PCARRD 1988)	Feasible	Significant Can cause severe and extensive damage to banana fruit with skin cracking, making fruit unmarketable (Pinese and Piper 1994)	Yes (WA only)
Elixothrips brevisetis (Bagnall 1919)	Banana rind thrips, Banana rust thrips	Yes Mau and Kessing (1993a)	Yes* NT, Qld (APPD 2005; Mound 2002)	Yes A polyphagous foliage feeder that usually feeds on leaves, flowers or stems, but is also known to damage fruit skins (Mau and Kessing 1993a)	Feasible	Significant Injured tissue turns dark brown, leaf tips wilt and curl, and flowers deform with many buds failing to open (Mau and Kessing 1993a)	Yes (WA only)
Thrips florum Schmutz 1913	Banana flower thrips; scab thrips	Yes Palmer and Wetton (1987); Nakahara (1994)	Yes WA, Qld, NSW (APPD 2005; Swaine and Corcoran 1975)				No
Thrips hawaiiensis (Morgan 1913)	Hawaiian flower thrips	Yes Nakahara (1994)	Yes WA, NT, Qld, NSW, Vic (APPD 2005; Miller et al 2002)				No
ARTHROPODA: ARACHNIDA: ACARI (Mites)							
Prostigmata: Tetranychidae Red spider mites							

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Eutetranychus orientalis (Klein 1936)	Citrus brown mite, oriental spider mite, oriental red mite	Yes Bolland et al (1998)	Yes WA, NT, Qld, NSW (APPD 2005; Halliday 1998; Walter et al 1995)				No
Oligonychus orthius Rimando 1962	Spider mite	Yes Corpuz-Raros (1989)	No Halliday (1998)	Yes Associated with *Musa* species (Bolland et al 1998)	Feasible	Significant *O. orthius* is a specialist grass feeder that is likely to damage crops such as sugar cane, sorghum and maize, as well as pasture grasses (Beard et al 2003)	Yes
Oligonychus velascoi Rimando 1962	Coconut spider mite	Yes Corpuz-Raros (1989); Cayme and Gapasin (1987)	No Halliday (1998)	Yes Associated with *Musa* species (Bolland et al 1998)	Feasible	Significant This spider mite causes damage to coconuts and possibly also mango in the Philippines (Cayme and Gapasin 1987)	Yes
Raoiella indica Hirst	Red palm mite	Yes Pena et al (2006)	No Halliday (1998)	Yes Although no data exists on within-plant distribution for bananas, it is found on fruits of coconut palm (Pena et al 2006),	Feasible	Significant Red palm mite is a serious pest of commercial palms and plants within the Musaceae and Zingiberaceae families (Pena et al 2006)	Yes
Tetranychus marianae McGregor 1950	Tropical red spider mite	Yes Bolland et al (1998)	Yes NT (APPD 2008)	Yes Associated with *Musa* species (Bolland et al 1998)	Feasible	Significant This spider mite has been recorded on many crop species and is considered a pest on several of these (Noronha 2006)	Yes (WA only)
Tetranychus neocaledonicus (André 1933)	Vegetable spider mite	Yes Corpuz-Raros (1989)	Yes WA, NT, Qld, NSW (APPD 2005; Flechtmann and Knihinicki 2002; Bolland et al 1998; Halliday 1998; Gutierrez and Schicha 1983)				No

Scientific name	Common name	Reference for presence in the Philippines	Reference for presence in Australia	Potential for being on hard green banana fruit	Potential for establishment and spread	Potential for consequences	Consider species further?
Tetranychus piercei McGregor 1950	Red spider mite, banana leaf mite	Yes McGregor (1950); Corpuz-Raros (1989)	No Flechtmann and Knihinicki (2002); Halliday (1998)	Yes Recorded on fruit as *T. urticae* and *T. lambi* only (Pinese and Piper 1994)	Feasible	Significant This polyphagous species has hosts that include most types of tropical fruits including: banana, palms, groundnut, papaya, sweet potato, castor bean, common bean, *Polygala paniculata*, and *Clitoria ternatea*. It is a serious pest in parts of Asia and Oceania (PHA 2004a)	Yes
Tetranychus urticae Koch 1836 Syn. = *Tetranychus cinnabarinus* (Boisduval 1867)	Two-spotted spider mite, carmine spider mite	Yes Corpuz-Raros (1989)	Yes WA, NT, SA, Qld, NSW, Vic, Tas (APPD 2005; Flechtmann and Knihinicki 2002; Halliday 1998)				No

Appendix 5. Moko

Scientific name

Ralstonia solanacearum (Smith 1896) Yabuuchi et al 1995, comb. nov. (IJSB 1996)
[Burkholderiales: Ralstoniaceae]

Synonyms

Pseudomonas solanacearum (Smith 1896) Smith 1914;
Bacillus solanacearum EF Smith 1896;
Burkholderia solanacearum (Smith 1896) Yabuuchi et al 1992 comb. nov. (IJSB 1993).

Common names

Moko disease; Moko; Moko disease of bananas; banana Moko; banana wilt; bacterial wilt; bacterial wilt of banana; bacterial wilt disease of banana; vascular wilt disease of banana.

In the Philippines, Moko disease on the cooking bananas Saba (ABB) and Cardaba (BBB) is generally known as Bugtok disease (Hayward 2006) and locally in Negros Orientale (Philippines) as either Tapurok (Zehr and Davide 1969) or Tibaglon (Molina 1996). The disease is known as Hereque in Venezuela (Buddenhagen 1961).

Races and biovars of *R. solanacearum*

Ralstonia solanacearum is a species complex traditionally classified into five races, based on host range (Buddenhagen et al 1962; French and Sequeira 1970; He et al 1983) and pathogenicity (Buddenhagen and Kelman 1964; Hayward 1964; French and Sequeira 1970; Thwaites et al 2000). Races are:

Race 1 affects a wide range of host plants with representatives in more than 50 plant families, especially the Solanaceae and Asteraceae, as well as diploid bananas (Cook et al 1989; Buddenhagen 1994; Hayward 2000)
Race 2 affects *Musa* and *Heliconia* species (Cook et al 1989; Buddenhagen 1994; Hayward 2000)
Race 3 affects potato, tomato and some solanaceous weeds (Buddenhagen 1994; Hayward 2000)
Race 4 it is pathogenic to *Zingiber officinale* (ginger) and a few other hosts, although this race was never formally designated by Pegg and Moffett (1971) and (Denny 2006)
Race 5 it is distinguished by pathogenicity to *Morus* spp. (mulberries) (He et al 1983).

South and Central American *R. solanacearum* race 2 strains causing Moko disease in dessert bananas, plantains and *Heliconia* spp. were further divided into five groups based on their colony morphology, virulence and transmission (French and Sequeira 1970), refer to Table 5.1. These types are SFR (small, fluidal, round), B (banana), D (distortion), H (heliconia) and A (Amazon basin) strain. Whilst some are pathogenic to both bananas and plantains (A, SFR, B and D types), others are only pathogenic to plantains, but not bananas (H type). Some groups are also highly aggressive (SFR, A, B), while the D type is less so. These groupings also considered the frequency of transmission by insects and persistence in soil (Thwaites et al 2000).

An alternative classification system (Hayward 1964, 1991, 1994 and 2000) differentiates six groups of strains (biovars) on the basis of their oxidation and/or utilisation of six key carbon sources (that is, maltose, lactose, cellobiose, mannitol, sorbitol and dulcitol), the utilisation of trehalose and the production of gas from nitrate.

Table 5.1 Characteristics of some strains within *R. solanacearum* race 2 pathogenic to *Musa* spp.

Based on: Thwaites et al (2000)[1]; Cook and Sequeira (1994)[2]; French and Sequeira (1970)[3] and Fegan and Prior (2005)[4]

Strain type [1]	Distribution [1, 2]	Characteristics [1]	Ecology [1, 4]	M L G[1, 3, 4]
SFR (small fluidal round)	Central America, Venezuela, Colombia, Caribbean	Small fluidal round, slight formazan pigment	Highly pathogenic; insect transmission high, soil transmission low	25, 28
B (banana rapid wilt)	Central and South America, Philippines	Large, elliptical colonies, slight formazan pigment	Highly pathogenic; insect transmission low (but higher for Bugtok disease in cooking bananas), soil transmission high	24
D (Distortion)	Costa Rica, Guyana, Surinam	As for B strain above	Low pathogenicity for banana/plantain/ *Heliconia* spp.	24, 25
H (Heliconia)	Costa Rica	As for B strain above	Pathogenic for plantain but not for banana	24
A (Amazon basin)	Peru	Near round, slight formazan pigment	Highly pathogenic; insect transmission high	25

Restriction fragment length polymorphorism (RFLP) groups are synonymous with multi-locus genotypes (MLGs) as used by Granada et al (1993) and Fegan (2005).

The race classification of strains does not form natural groupings as shown by recent DNA-based diagnostic work, including the phylogenetic classification of Fegan and Prior (2006) that has superseded the race classification. The biovar classification can be related in part to the DNA-based classification and is still used as a guide to the diversity of strains in a particular country or locality.

Molecular genetics of *R. solanacearum*

Restriction fragment length polymorphism (RFLP) analysis determined that strains belonging to the *R. solanacearum* species complex can be divided into two divisions that closely reflect their origins in the Old and New World, respectively (Cook et al 1989; Cook and Sequeira 1994) – refer to Table 5.2. Division 1 (Old World) comprises strains belonging to the biovars 3, 4 and 5, while Division 2 (New World) comprises strains belonging to biovars 1, 2 and N2 (Hayward 2000). Strains that cause Moko disease in *Musa* spp. and bacterial wilt disease in *Heliconia* spp. belong to biovar 1. This dichotomy of *R. solanacearum* has been confirmed using various other molecular methodologies (Seal et al 1992; Gillings et al 1993; Taghavi et al 1996). Informally, divisions are regarded as subspecies (Hayward 2000).

Two more genetically distinct groups within the *R. solanacearum* complex were identified using DNA sequencing techniques. These comprise '*Pseudomonas celebensis*' the Blood Disease Bacterium (BDB), *Ralstonia syzygii* (Sumatra disease of cloves) (Fegan et al 1998), and a group of strains originating in Africa (Poussier et al 2000). It is therefore understood that the *R. solanacearum* species complex can be divided into four geographically distinct groups (or phylotypes) and the following hierarchical classification system has been proposed by Fegan and Prior (2005):

Phylotype I: equivalent to Division 1 (Cook and Sequeira 1994);
Phylotype II: equivalent to Division 2 (Cook and Sequeira 1994); this group includes the pathogen causing Moko disease of *Musa* spp. and bacterial wilt of *Heliconia* spp.;
Phylotype III: strains primarily of African (including surrounding islands) origin;
Phylotype IV: strains that have been found in Australia and Japan, including the close relatives of *Ralstonia solanacearum*, such as *R. syzygii* (Sumatra disease of cloves and '*Pseudomonas celebensis*' (Blood Disease Bacterium).

Later work by Guidot et al (2007) and Castillo and Greenberg (2007) has confirmed the genetic

diversity of this species.

RFLP fingerprinting technique, developed by Cook et al (1989, 1991) and Cook and Sequeira (1994), recognised 46 RFLP groups of which three are pathogenic to *Musa* spp. These consist of the distinctive genotypes RFLP/multi-locus genotype (MLG) 24, RFLP/MLG 25 and RFLP/MLG 28 (Cook et al 1989; Fegan 2005) – refer to Table 5.2. Granada et al (1993) added five more MLGs (that is, MLG 47–51) isolated from plantain varieties in Colombia; none of those resembled MLG 24, which is present in the Philippines (Fegan 2002; Fegan and Prior 2006). Fegan (2002, 2005) established that *R. solanacearum race* 2 is clustered into three distinguishable sequence variants designated as sequevars 3, 4 and 6 which equate with MLG 24, MLG 25 and MLG 28, respectively, and the newly determined genotype sequevar 24 Table 5.3. Fegan's research was based on sequencing the virulence-associated endoglucanase gene as well as the genomic fingerprinting technique (that is, *rep*PCR). Intra-sequevar differences of sequevars 3, 4 and 6 were described as being 'clearly unique' (Fegan 2002).

Table 5.2 Relationships of the *R. solanacearum* complex

The description of the relationships include phenotypic (biovars and races) and genotypic (RFLP analysis and phylotyping) schemes – after Gillings and Fahy (1994) as mentioned in Fegan (2005)

<table>
<tr><th>Species</th><th colspan="19">R. solanacearum species complex</th></tr>
<tr><th>Phylotype</th><th colspan="6">Phylotype I</th><th colspan="6">Phylotype II</th><th colspan="2">Phylotype III</th><th colspan="5">Phylotype IV</th></tr>
<tr><th>Distribution</th><th colspan="6">Asia, Africa, South America</th><th colspan="6">America
(Race 3 - worldwide)</th><th colspan="2">Africa</th><th colspan="5">Indonesia, Japan, Australia</th></tr>
<tr><td>Biovars</td><td colspan="3">3</td><td colspan="2">4</td><td>5</td><td colspan="2">2T</td><td colspan="2">1</td><td colspan="2">2</td><td>2T</td><td>1</td><td>2T</td><td>1</td><td>2</td><td>P. sy.</td><td>BDB</td></tr>
<tr><td>Division</td><td colspan="6">Division 1</td><td colspan="6">Division 2</td><td colspan="2">?</td><td colspan="5">?</td></tr>
<tr><td rowspan="3">Multi-locus genotypes (MLGs)</td><td>8</td><td>9</td><td rowspan="3">11</td><td>15</td><td>21</td><td>19</td><td>29</td><td>30</td><td>1</td><td>2</td><td>24</td><td>26</td><td rowspan="3" colspan="2">?</td><td rowspan="3" colspan="5">?</td></tr>
<tr><td>10</td><td>12</td><td>16</td><td>22</td><td>—</td><td>31</td><td>32</td><td>3</td><td>4</td><td>25</td><td>—</td></tr>
<tr><td>13</td><td>14</td><td>17</td><td>23</td><td>20</td><td>33</td><td></td><td>5</td><td>6</td><td>28</td><td>27</td></tr>
<tr><td>Races</td><td colspan="4">1</td><td>4</td><td>5</td><td colspan="4"></td><td>2</td><td>3</td><td colspan="2">1?</td><td colspan="5">1?</td></tr>
</table>

Table 5.3 Relationships between genetic findings using RFLP fingerprinting and endoglucanase gene sequence analysis

Source: after Fegan and Prior (2005), and Fegan (2005)

Multi-locus genotype (MLG)[1]	MLG 24	MLG 25	MLG 28	Undetermined
Sequevar[2]	3	4	6	24

[1]MLG, determined by restriction fragment length polymorphism (RFLP) fingerprinting technique
[2]A sequevar is a group of strains with a highly-conserved endoglucanase gene sequence

Strains pathogenic to *Musa* spp. phenotypically belong to race 2 as characterised by Buddenhagen et al (1962) and biovar 1 under the classification of Hayward (1964), and genetically consist of the distinctive multi-locus genotypes MLG 24, MLG 25 and MLG 28, which all belong to Division 2 of *R. solanacearum* (Cook et al 1989; Cook and Sequeira 1994). Recently, further Moko causing strains, infectious to bananas in Brazil, were detected using molecular analysis of the endoglucanase virulence gene. Thus far, no RFLP analysis has been undertaken for the newly found sequevar and therefore its MLG equivalence is unknown (Fegan 2005; Fegan and Prior 2006). A Bugtok-causing isolate from the Philippines was recently identified as *R. solanacearum* MLG 24 (Fegan and Prior 2006). Based on the currently available information, *R. solanacearum* race 2 strains in the Philippines are of low variability and, based on molecular tests, considered monomorphic (Raymundo et al 2005; Fegan and Prior 2006).

In Martinque (French West Indies), strains of *R. solanacearum* with a new pathogenic potential have been obtained from anthurium and some other hosts which, using the molecular classification of Fegan and Prior (2005), belonged to phylotype II/ sequevar 4, which includes strains causing Moko disease. However, the new strains were not pathogenic to banana (Wicker et al 2007).

Hosts

About 300 species representing about 50 plant families have been described as hosts of *R. solanacearum*; they include solanaceous vegetable crops, banana, ginger, custard apple, peanut, *Eucalyptus* spp and many other crop plants and weeds (Kelman 1953; Hayward 1991). Of these, *R. solanacearum* race 2/biovar 1 (the banana race) produces typical wilt symptoms on a narrow range of host plant species under natural conditions. *Musa* spp. (banana and plantain) and *Heliconia* spp. exhibit externally visible symptoms in the form of moderate to severe wilt depending on host genotype, pathogen strain and environmental conditions (Buddenhagen 1960, 1961; Stover 1972; Woods 1984; Molina 1996). Whilst symptom expression generally occurs rapidly under ideal conditions, banana and heliconia plants may also remain symptomless for many months (Stover 1972).

Diploid, triploid and tetraploid *Musa* spp. are considered hosts of *R. solanacearum* race 2 (Buddenhagen 1961; Stover 1993; Soguilon et al 1995; Thwaites et al 2000). There are three diploid *Musa* species – *M. fitzalanii* (Australimusa), *M. jackeyi* (Australimusa), and *M. acuminata* ssp. *banksii* (Eumusa) – endemic to Australia. All of these species are associated with isolated rainforest environments in tropical northern Australia (Ross 1987; Daniells 1991). The distribution statuses of *M. fitzalanii* (Australimusa) and *M. jackeyi* (Australimusa), are listed on the World Conservation Monitoring Centre website[2] as extinct and rare, respectively. *M. acuminata* ssp. *banksii* represents the most common of the native species, and small and scattered populations are found in between Ingham and Cape York (Daniells 1991).

All *Heliconia* spp. are considered hosts of *R. solanacearum* race 2 (Buddenhagen 1960, 1961; Sequeira and Averre 1961; Kastelein and Gangadin 1984; Ferreira et al 1991; Assis et al 2005). Buddenhagen (1960) published the first report of the pathogen infecting *H. caribaea* and *H. latispatha* in Coto, Costa Rica. Sequeira and Averre (1961) reported infection of *H. latispatha*, *H. acuminata* and *H. imbricata* under natural conditions also in Coto, Costa Rica. Kastelein and Gangadin (1984) reported bacterial wilt in *H. bihai* in the Cottica river basin in East Surinam. Bacterial wilt was reported on *H. psittacorum* as well as *H. rostrata* in Hawaii (Ferreira et al 1991; Yu et al 2003). Assis et al (2005) reported bacterial wilt on *H. bihai*, *H. caribaea, H. humilis, H. nickeriensis, H. psittacorum* cv. Lady Di, cv. Red Opal, cv. Sassy, cv. Strawberries and Cream, *H. psittacorum* x *H. spathocircinata* cv. Golden Torch, *H. rauliniana* and *H. wagneriana* plants in the state of Pernambuco, Brazil.

In addition to bananas and heliconias, the pathogen is reported to be associated with a range of other plant species, predominantly weeds (Berg 1971). It may occur in the root zone (rhizosphere) of certain native flora (Wardlaw 1972) or cause infection in some of these plant species either without producing external symptoms or producing very subtle symptoms (Belalcazar et al 1968; Granada 2002); hence their description in the literature as symptomless or asymptomatic carrier hosts or latently infected hosts.

Belalcazar et al (1968) found four species of weeds – *Brassica campestris* (field mustard), *Datura stramonium* (thorn apple), *Solanum caripense* and *S. nigrum* (blackberry nightshade) – in Colombia that could carry the banana strain without showing external symptoms but were susceptible when artificially inoculated. French and Sequeira (1970) reported that *R. solanacearum* race 2 isolates from Colombia and Peru rapidly killed inoculated plants of pepper, potato and tomato and were pathogenic to tobacco. Berg (1971) listed the following weed hosts of the SFR strain of *R. solanacearum* race 2 in Honduran banana farms: *Asclepias curassavica, Cecropia peltate, Piper auritum, P. peltatum, Ricinus*

[2] Website: http://www.unep-wcmc.org

communis, Solanum hirtum, S. nigrum, S. umbellatum, S. verbascifolium, and *Xanthosoma roseum*. He also reported that two additional species, *Physalis* sp. and *Solanum torvum*, were susceptible in artificial inoculation experiments. Isolates from all species listed above were pathogenic when artificially injected into young, potted plants of banana v. Valery (AAA) (Berg 1971).

Granada (2002) reported that a survey of plantations in the Quindío State of Columbia showed that the Moko bacterium may be carried by seven asymptomatic weed hosts: *Bidens pilosa* (cobbler's-pegs), *Browalia americana, Commelina* spp., *Emilia sanchifolia*, *Phyllanthus corcavadensis, Pilea hyaline* and *Solanum nigrum* (black nightshade). Fegan (2002) detected *R. solanacearum* race 2 (MLG 28) in a naturally infected tomato plant from Brazil. The bacterium was identified using endoglucanase gene sequence analysis. However, Fegan recognised that this methodology may not be sensitive enough to distinguish between Moko causing and non-Moko causing strains, as the analysed DNA sequence varied in no more than four base pairs over a length of about 750 base pairs.

In 2002, the Philippine authorities informed Biosecurity Australia that there were three alternative Moko hosts: *Brassica campestris, Heliconia* spp. and *Solanum verbascifolium* (BPI 2002b). This information was taken from the Host Index of Plants in the Philippines. None of the literature from the Philippines accessed to date provides information about the identity or importance of weed hosts in the epidemiology of Moko. However, there is consensus amongst authorities that weed control is an essential component for the management of Moko bacterial wilt and constitutes a recommended practice in the Philippines (PCARRD 1988; BPI 2001).

Álvarez et al (2008) used histological studies to determine three categories of host plants depending on whether xylem was colonised or not; susceptible hosts, tolerant hosts and non-hosts. The first two categories include plants infected by high densities of the pathogen in xylem vessels at the root level, while invasion of xylem at the middle part of the stem is heavy in susceptible plants and strongly limited in tolerant plants. *Solanum dulcamara* (Bittersweet nightshade) is a symptomless carrier of some potato strains of *R. solanacearum* and therefore is a tolerant host, although wilting has been occasionally reported. The last group refers to non-host plants and includes species in which no xylem invasion was observed though some external contamination of the rhizoplane and rare cortical infection pockets may occur. The terms used to describe plant susceptibility in the pest risk analysis are equivalent to those used by Álvarez et al (2008). A symptomatic host is equivalent to a susceptible host; an asymptomatic host is equivalent to a tolerant host; and a plant not colonised is equivalent to a non–host.

Distribution

Table 5.4 Distribution list for *R. solanacearum* race 2

Country	References
Belize	Black and Delbeke (1991); Phelps (1987)
Brazil	Lehmann-Danzinger (1987); Fegan (2005)
Colombia	Lehmann-Danzinger (1987); Phelps (1987)
Costa Rica	Lehmann-Danzinger (1987); Phelps (1987)
Ecuador	Lehmann-Danzinger (1987)
El Salvador	Lehmann-Danzinger (1987)
Grenada	Lehmann-Danzinger (1987); Phelps (1987)
Guatemala	Lehmann-Danzinger (1987); Phelps (1987); Sanchez Perez et al (2008)
Guyana	Lehmann-Danzinger (1987); Phelps (1987)
Honduras	Lehmann-Danzinger (1987); Phelps (1987)
India	Gnanamanickam et al (1979); Fegan (2005)
Jamaica	ProMed (2004)
Mexico	Lehmann-Danzinger (1987)
Nicaragua	Lehmann-Danzinger (1987); Phelps (1987)
Panama	Lehmann-Danzinger (1987); Phelps (1987)
Peru	Lehmann-Danzinger (1987)

Country	References
Philippines	Lehmann-Danzinger (1987)
USA (Hawaii)[1]	Diatloff et al 1992; Sewake and Uchida (2005)
St. Vincent and the Grenadines	http://www.ippc.int (Moko disease declaration)
Surinam	Lehmann-Danzinger (1987); Phelps (1987)
Trinidad	Lehmann-Danzinger (1987); Phelps (1987)
Venezuela	Lehmann-Danzinger (1987); Phelps (1987)

[1]*In Hawaii on heliconia only*

Bugtok disease of cooking bananas was first reported in Mindanao region in the early 1950s (Soguilon et al 1994b) and is also found in Davao City, the three Davao provinces (Davao del Norte, Davao Oriental and Davao del Sur) and in Bukidnon (Soguilon et al 1990). Bugtok disease is widely distributed on cooking bananas throughout the Philippines, including Mindanao Island (BPI 2002b).

Ralstonia solanacearum race 2 has been reported only within tropical regions between latitudes 21.3° N (Hawaii) and 17° S (Peru) of the Equator (see Table 5.4). The tropics are defined as the region between 23º 27' N – 23º 27' S of the Equator.

Regions where Moko has established are characterised by lowland, humid tropics (Kelman 1953; Buddenhagen 1961; Stover and Simmonds 1985; Jeger et al 1995) with the exception of Colombia where the pathogen occurs also at higher altitude (Belalcazar et al 1968; Belalcazar et al 2004; Granada 2002; Lehmann-Danzinger 1987).

There are no confirmed instances where *R. solanacearum* race 2 has established in a subtropical location (Buddenhagen 1961, 1986; Hayward 2000).

Reports from Guadeloupe are incorrect and details of *R. solanacearum* race 2 from Africa are limited and cannot be substantiated (Thwaites et al 2000). The pathogen is present within the Amazon region in northern, equatorial Brazil, Peru, Ecuador, Colombia, Guyana and Surinam (Stover 1972; Muirhead 2003).

Biology

Ralstonia solanacearum is a Gram negative, aerobic, non-spore forming, rod-shaped bacterium (Kelman 1953) that produces extracellular polysaccharide (EPS) but lacks a discrete capsule; possesses polar pili (Strom and Lory 1993) and 2–3 flagella (Clough et al 1997); minimum growth temperature of 8–10 °C, optimum at 32–35 °C, maximum ~40 °C, thermal death point ~52 °C (Kelman 1953); minimum pH 6.0, optimum pH 6.6, maximum pH 8.0; growth is retarded by 0.5% NaCl, very weak at 1.0% and prevented by 2% NaCl (Bryan 1915; Kelman 1953); non fluorescent (belonging to *Pseudomonas* rRNA group II (Palleroni 1984). Motility via flagella is only observed at high cell densities (10^7–10^9 mL^{-1}) (Clough et al 1997). Polar pili enable the bacterium to migrate over solid surfaces by means of 'twitching' propulsion (Liu et al 2001).

Plant parts affected

The bacterium infects banana plants at all growth stages and symptoms of the disease vary depending on the age of the plant, physiology of banana cultivars, mode of transmission of the pathogen and the strain of the Moko pathogen causing the infection (Stover 1972; Thwaites et al 2000). Symptomatic hosts such as *Musa* and *Heliconia* will die from infection with the pathogen (Wardlaw 1972; Assis et al 2005).

The root, rhizome (commonly referred to as a corm), pseudostem, leaf, flower and fruit of hosts can show symptoms of *R. solanacearum* race 2 via systemic infection of the vascular bundles (Stover 1972). In Bugtok disease of cooking bananas in the Philippines, there is fruit infection following insect transmission. Infection through the inflorescence in Cavendish is mainly the result of systemic infection (Hayward, 2006).

Seed infection has been mentioned in the literature (Buddenhagen 1961; French and Sequeira 1968; Black and Delbeke 1991; Molina 1996), but these references refer to the vegetative plant parts used for propagation, that is, ‘corms’ or ‘suckers’. True seed transmission of *R. solanacearum* race 2 in *Musa* spp. is not reported in the literature. However, seed transmission is known to occur in a number of plant species affected by races 1 and 3 (Hayward 1991, 2000; Momol et al 2005).

Location of bacteria

Ralstonia solanacearum race 2 causes systemic infection of the xylem and the apolplast of associated parenchyma tissue of its hosts. The pathogen is unable to invade unwounded plant surfaces and requires access to the vascular bundles via exposed vascular tissue (Kelman 1953; Lehmann-Danzinger 1987; Swanson et al 2005). Within hosts, large numbers of bacteria are concentrated in the primary and secondary xylem vessels, with some colonisation of the intercellular spaces, and some leakage into the adjacent parenchyma cells (Stover 1972; Sequeira 1993). *Ralstonia solanacearum* race 2 causes blockage of the vascular system, including that to fruit (Buddenhagen 1994). Dry rot of the fruit is considered to be a host response to blockage of xylem vessels by bacterial cells and polysaccharides produced by the bacteria and subsequent alteration of water movement (Stover 1972; Wardlaw 1972; Genin and Boucher 2002).

The bacterium divides by transverse binary fission but exact information on the generation time *in planta* is lacking. Bacterial ooze expressed from the stems of inoculated tomato plants, for example, may contain in the order of one billion to 10 billion colony forming units (cfu) per mL, depending on the susceptibility of the cultivar and growth conditions. The pathogen multiplies in the xylem vessels of the host plant and will eventually be shed through the roots of the host into the soil (Kelman and Sequeira 1965; Schell 2000; Swanson et al 2005).

Ralstonia solanacearum race 2 has been isolated from the pedicel, pulp and fruit tip of symptomless infected Cavendish banana fruit when harvested at the mature hard green development stage in the Philippines (Soguilon et al 1994a; Soguilon 2003a). Isolations of *R. solanacearum* are made most successfully from the vascular bundles of the pseudostem and peduncle where bacterial populations may occur in high numbers. The most numerous vascular bundles in the fruit occur in the fibrous tissue of the peel.

Infected fruit, peduncles or fruit stalks immersed in water may not always show the presence of bacterial ooze (see definition in List of Terms). Bacteria were only obtained when infected and detached male axes (that is, the part of the peduncle that connects the upper female flowers with the lower male flowers) and male inflorescences were incubated for 3–14 days or until exudates from the cut ends were visible (Soguilon et al 1994a).

In later work, isolates of the pathogen from Moko bacterial wilt of Cavendish bananas and Bugtok fruit rot of cooking bananas were artificially inoculated into corms or peduncles of Cavendish banana plants (Soguilon 2003a). In the case of corm tissue, 10^8 bacterial cells in 1 mL water were injected into the corm tissue of plants ready for debelling. In the case of peduncle tissue, 10^2, 10^5 or 10^8 bacterial cells in 1 mL of water were sprayed onto the freshly cut surface of a peduncle at the time of debelling. After incubation for 9–11 weeks, two fingers were sampled from each hand of each bunch on inoculated plants and the pedicel, fruit tip and pulp tissue samples were cultured on selective media. The proportion of hands from which *R. solanacearum* race 2 was isolated was determined. There were no significant differences between the strains of the bacterium that were used in the experiments, and results were pooled.

The results summarised in Figure 5.1 indicate that bacteria were isolated from the pedicel tissue of up to 13% of hands, from the fruit tip tissue of up to 13% of hands, and from the pulp tissue of up to 3.5% of hands when peduncle tissue was inoculated. The results for 10^2 and 10^5 cells per inoculated site were similar, whereas the pathogen was recovered from fewer hands than when peduncles were inoculated with 10^8 cfu per inoculation site. In the case of plants inoculated by corm injection, the bacteria were recovered from a maximum of only 3% of hands. Significantly, Soguilon (2003a) noted that all inoculated plants displayed vascular browning in the peduncle or cushion tissue when fruit was examined 10–13 weeks after inoculation.

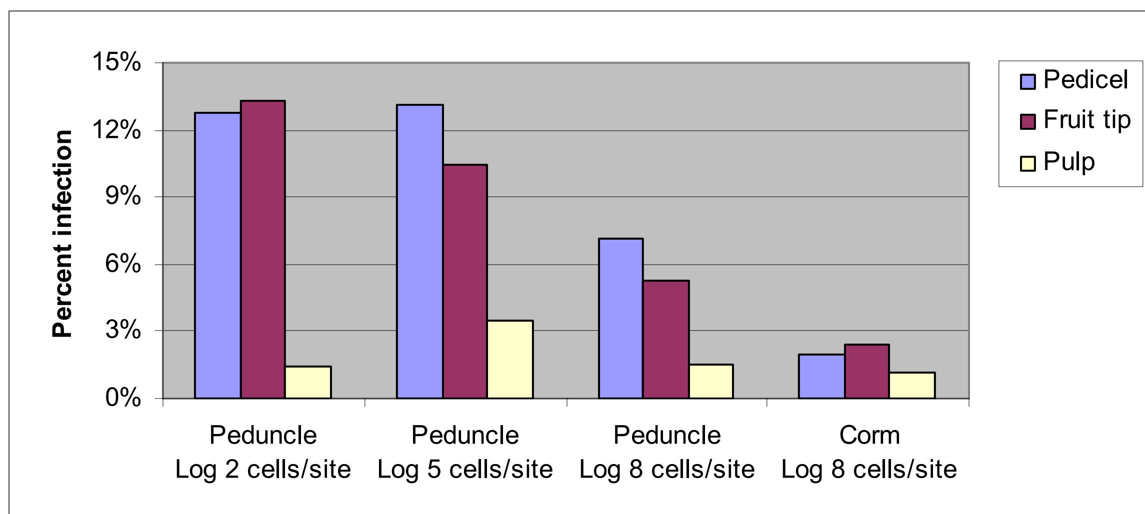


Figure 5.1 Recovery of *R. solanacearum* race 2 from banana fruit

Percentage of Cavendish banana hands from which R. solanacearum race 2 was recovered from pedicel, fruit tip or pulp tissue of mature, hard green Cavendish banana fruit 10–13 weeks after inoculating the peduncle or corm with 10^2 (Log 2), 10^5 (Log 5) or 10^8 (Log 8) bacterial cfu per inoculation site. Source: derived from Soguilon (2003a)

Soguilon (2003a) did not report on the isolation of *R. solanacearum* race 2 from individual fingers sampled in the experiments, so it is not possible from this work to estimate the proportion of fruit clusters or fingers that became infected as a result of the inoculation.

Availability of entry points

Ralstonia solanacearum race 2 requires entry holes and exposure to the vascular bundles in order to cause infection in its hosts (Kelman 1953). The pathogen is unable to cause infection of intact above ground plant surfaces, such as the peel of Cavendish bananas (Soguilon 2003b) or leaves of *Capsicum annuum* (Momol et al 2005). Natural plant openings, such as stomata, are not involved in the infection pathway (Kelman 1953).

The most susceptible natural sites for the pathogen to enter its hosts are the fresh abscission scars of floral bract and flowers (Buddenhagen and Elsasser 1962; Buddenhagen 1994), root-to-root contact (Kelman and Sequeira 1965) via points of secondary root emergence (Sequeira 1993; McGarvey et al 1999), wounds created from injuries caused by insect damage, and/or by mechanical means (Sequeira 1958; Lehmann-Danzinger 1987).

Inoculum dose

Edible bananas have no resistance genes to *R. solanacearum* race 2 (Raymundo et al 1998; Sequeira 1998). Although the minimum infective dose for *R. solanacearum* race 2 is not known, research on the related disease, brown rot of potato, caused by *R. solanacearum* race 3/biovar 2, demonstrated that a minimal dose of between 1–10 cfu of *R. solanacearum* strain 1609 was able to cause wilting in 12–75% of stem inoculated tomato plants (van Overbeek et al 2004). These results are consistent with research undertaken on the related *R. solanacearum* race 3 strain, where almost all geranium plants died after direct stem inoculation with only a few hundred cfu each (USDA 2004).

In contrast, when plant parts such as *Musa* roots remained unwounded, extremely high numbers of *R. solanacearum* bacteria were required to cause infection (Kelman and Sequeira 1965). The authors demonstrated that the pathogen was incapable of digesting its way through cortical cells to reach the vascular tissue when bacterial suspensions (of undefined quantity) were placed directly onto loci free of potential entry holes, such as points of secondary root emergence. Similar results were obtained in an experiment with the related pathogen *R. solanacearum* biovar 2/race 3 strains 1609, where tomato plants remained uninfected when soil was inoculated with 5mL of 10^6 or more *R. solanacearum* strain

1609 cells (van Overbeek et al 2004). These results are also consistent with frequent field observations where the incidence of bacterial wilt increases with an increase of wounded hosts (Sequeira 1993).

As demonstrated by the artificial inoculation experiments of *R. solanacearum* race 3, as little as between one and 10 bacterial cfu could be sufficient to cause infection. It is therefore expected that a similar small inoculum load of *R. solanacearum* race 2 could cause infection when bacterial cells would come into direct contact with exposed vascular tissue of their hosts, regardless of wounds being below or above ground. However, the probability of this occurring in the field is low because of the adverse biotic and abiotic factors. The pathogenicity and viability of one or a small number of bacterial cells is expected to be under immense stress as its metabolic functions are poorly protected from its immediate environment (Monier and Lindow 2003; Álvarez 2005). The infection and colonisation potential of *R. solanacearum* is associated with the aggregation of large numbers of bacterial cells and the presence of large amounts of exopolysaccharides (EPS). EPS are thought to provide binding sites for the colonisation of roots (Aroud-Razou et al 1998).

Incubation period

Reports on the incubation period, (that is, time elapsed between inoculation and expression of external visible disease symptoms), vary from less than one week to 24 weeks or more, depending on the maturity of the infected plant, route of infection, method of inoculation and environmental conditions, particularly the incubation temperature and to some extent the relative humidity (refer to Table 5.5). The incubation period in young actively-growing plants is shorter than in mature plants (Sequeira 1958). In the former, it produces symptoms in two to four weeks (Buddenhagen 1961, 1994), whereas in mature plants the incubation period may be greater (refer to Table 5.5). The incubation period in banana fruit has been reported as three weeks (Sequeira 1958), but it is reasonable to assume that this period could be longer, given that a wide range of incubation periods have been recorded in banana plants.

Table 5.5 Incubation period of *R. solanacearum* in *Musa* spp. (external symptom expression)

Incubation period	Plant type and growth stage	Banana cultivar (genotype)	Strain of race 2	Method of inoculation	Comment	Reference
1 month	Half grown plants	Moko (Bluggoe) plantain (ABB)	B#	Injecting bacterial suspension into one of the upper leaves	The injected plant was dead within 7 weeks and suckers died within about four and half months	Rorer (1911)
14–19 days		Red banana	B#	Smearing bacterial growth on a young leaf and pricking 6–8 times into the petiole	The inoculated leaves broke down and within the next 2 weeks the whole plant was dead	Rorer (1911)
17–24 days		Red banana	B#	Smearing bacterial growth on a young leaf and pricking 6–8 times into the petiole	The inoculated plants were dead within 7 weeks	Rorer (1911)
7 weeks		Dwarf banana (AAA)	B#	Smearing bacterial growth on a young leaf and pricking 6–8 times into the petiole	Inoculated plants died in 13 weeks, in addition, many suckers were diseased or even dead (also see below[1])	Rorer (1911)
7 weeks		Banana	B#	De-suckering with a machete, which was infested by piercing through a diseased pseudostem	The bacteria moved rapidly through the plant when a young leaf was pruned; with all plants showing symptoms within 7 weeks. It moved down the petiole very slowly when fully matured leaves were pruned.	Sequeira (1958)
3 weeks	Mature plants, peduncles	Banana	B#	De-budding with an infested knife	100% of the treated plants developed symptoms	Sequeira (1958)

Incubation period	Plant type and growth stage	Banana cultivar (genotype)	Strain of race 2	Method of inoculation	Comment	Reference
7 weeks	Mature plants, peduncles	Banana	B#	De-leafing a young and actively growing leaf with an infested knife	100% of the treated plants developed symptoms (also see below[1])	Sequeira (1958)
30 days or more	Young and mature plants	Banana	B#	Pruning young suckers and smearing the pruning wounds with soil from a heavily Moko-infested area	32.6% of the treated plants developed symptoms in 30 days and 6.6% of the suckers belonging to these plants developed symptoms within this time	Sequeira (1958)
7–45 days	3 ft tall young plants	Gros Michel (AAA)	B#	Injecting bacterial suspension into the pseudostem about 15 cm above the soil level	Most of the 'normal' isolates produced rapid wilting symptoms in 7 days – plants inoculated with a 'distortion' strain showed symptoms within 45 days	Buddenhagen (1960)
2–4 weeks	Young regrowth suckers		B#	De-suckering with contaminated knife	Symptoms progressed rapidly on young, actively-growing plants; most leaves collapsed within a few days to a weeks after initial wilting symptoms appeared	Buddenhagen (1961)
8–29 days or more	1 month old plants	Gros Michel (AAA)	B (ex-banana), B (ex-heliconia)	Injecting bacterial suspension into the pseudostem base	The isolates from banana caused a dramatic quick wilt, as compared with slow wilt caused by isolates from heliconia	Sequeira and Averre (1961)
1 month	Immature fruit	Saba (ABB)	B	Injecting bacterial suspension into the fruit		Zehr and Davide (1969)
4–8 days	15 cm tall plants	*M. balbisiana* (BB)	B, D, SFR, T	Piercing pseudostem 2–3 cm above the soil line	Incubation temperature ranged from 30–33 °C at night to 30–40 °C in the day time	French and Sequeira (1970)
6 weeks–3 months or more	Mature plants	Banana		Pruning suckers with contaminated machete	40% of the mats showed symptoms after 70 days and 60% after 90 days[2]	Stover (1972)
5 weeks	Young plants			Injecting bacterial suspension in the pseudostem or the rhizome or placing extract from infected peduncle on damaged parts of rhizomes		Power (1976)
7–10 days	Young plants with 4 or more expanded leaves	Giant Cavendish (AAA)		Pouring bacterial suspension into the exposed and injured lateral roots 5–10 cm from either the base or corm		Rillo (1979)
8–24 weeks or more	Young, 1 m high plants		B, SFR	Pouring bacterial suspension over the cut stub after pruning the fourth newest leaf at the junction with the pseudostem		Woods (1984)
2 weeks	Young plants, 3 month old	Bluggoe (ABB)	SFR	Cutting roots 1 cm below the root neck and then dipping them in bacterial suspension or injecting bacterial suspension into the pseudostem		Lehmann-Danzinger (1987)
Up to 3 months	Mature plants		B			Lehmann-Danziger (1987)
4 months	Mature plants	Abuhon (BB)	B#	Injecting pseudostem with bacterial suspension	Symptoms were seen in the fruit four months after inoculation	Soguilon et al (1994a)
6–12 days	Young tissue cultured plants	Cardaba (BBB)	B#	Injecting pseudostem with bacterial suspension		Soguilon et al (1994b)

Incubation period	Plant type and growth stage	Banana cultivar (genotype)	Strain of race 2	Method of inoculation	Comment	Reference
6 days	Young plants, suckers about 80 cm tall	Cavendish	B[#]	Injecting bacterial suspension into the base		Soguilon et al (1994b)
Not recorded	Mature plants	Abuhon (BB)	B[#]	Injecting bacterial suspension into newly emerged, punctured inflorescence	Typical Bugtok symptoms appeared in fruit and bracts covering the male flowers	Soguilon et al (1994b)
More than 13 weeks	Mature plants	Cavendish (AAA)	B	Sprayed bacterial suspension onto fresh peduncle wounds immediately after de-belling. Sprayed surface covered with plastic film for 48 hours	None of the inoculated plants expressed external symptoms at the end of the 13-week observation period. All test plants manifested varying degrees of vascular discolouration in peduncle	Soguilon (2003a)

[1] In two mats, suckers were symptomless until they reached maturity; they produced very poor bunches, some of the fingers being black and rotten. Internally, the vascular bundles in the stem, fruit stalk and fingers of these plants were discoloured and filled with bacteria.

[2] When a chisel type tool was used to remove unwanted suckers and mother plants close to or below ground level, some rhizomes were infected, but infection remained localised. The fact that some infections remain latent or do not become systemic for long periods complicates detection and control.

[#] It is presumed the B strain was used in these experiments.

Symptoms

The earliest visible symptoms caused by root infection on symptomatic hosts are signs of yellowing and wilting of the three youngest leaves, followed by subsequent yellowing and wilting of older leaves. Leaves of infected plants eventually become necrotic and collapse at the junction of the lamina with the petiole (Stover 1972; Diatloff et al 1992; Buddenhagen 1994). The whole plant eventually dies. Rapid wilting may be accompanied by bending of the plant so that it snaps off at the base close to the ground. Young suckers may wilt without showing the foliar symptoms of yellowing and necrosis. Rapidly growing suckers of infected mats are generally blackened and deformed. Blackened and dead male flower buds and peduncles with vascular discolouration advancing into the pedicel and fruit are indicative of Moko disease on commercial triploid varieties (Stover 1972). Fruit development is arrested and fingers may ripen prematurely, become discoloured and/or split and eventually rot (Sequeira 1958; Buddenhagen 1961; Stover 1972; Thwaites et al 2000). Bunch infection is often indicated by a small number of yellow distorted fruits (that is, fingers) appearing on an otherwise green bunch. The pulp of infected fruit becomes discoloured and may eventually exhibit a firm dry rot (Buddenhagen 1994).

Internally, Moko infection causes vascular discolouration irrespective of whether external disease symptoms develop (Rorer 1911; Ashby 1926; Martyn 1934; Sequeira 1958; Buddenhagen 1961; Power 1976; Kastelein and Gangadin 1984; Jeger et al 1995; Soguilon 2003a). The vascular tissues of all infected plant parts (including the corm, pseudostem, peduncle and fruit) are filled with bacteria and progressively change colour from cream or yellow to brown or black. Shortly after tissues are cut, creamy white drops of bacterial ooze exude from transverse sections of infected leaf petioles, stems or peduncles. Discolouration of vascular bundles in the young tissue at the centre of the pseudostem may be observed throughout the plant (Thwaites et al 2000). In some instances infection can cause the development of cavities in the pseudostem, corm and peduncle, due to disintegration of the vascular tissue. Continual discolouration of the vascular bundles from the corm to the peduncle and into some fruit is indicative of soilborne Moko infection (Wardlaw 1972).

In contrast, asymptomatic carrier hosts, which can also be regarded as tolerant hosts (Álvarez 2008), appear visually healthy externally, although they often display internal discolouration of the vascular tissue within roots and stems (Berg 1971). Vascular discolouration is indicative of the colonisation of the vascular system by the pathogen, but in the case of asymptomatic host carriers, not at a sufficient level to induce externally visible wilt symptoms. Visible symptoms are, at the most, subtle under natural conditions (Belalcazar et al 1968; Berg 1971; Granada 2002). External symptoms may develop

following artificial inoculation and incubation under favourable environmental conditions (Belalcazar et al 1968; Berg 1971).

Disease cycle

Figure 5.2 illustrates the key pathways in the disease cycle of Moko infection caused by *R. solanacearum* race 2 on *Musa* plants. These pathways are:

- soil to root to corm pathway; or
- mechanical transmission by cutting implements; or
- insect transmission, to either
    - natural wounds caused by abscising bracts and male flowers on the inflorescences; or
    - wounds caused on the plant by standard plantation operations.

The epidemiology of Moko disease in Cavendish (AAA genotype) bananas differs markedly from that observed in cooking bananas (ABB or BBB genotype). In Cavendish bananas, *R. solanacearum* race 2 is most commonly transmitted by the mechanical means of contaminated (unsanitised) machetes or knives used in routine field operations, as well as through exchange of infected planting material. Table 5.6 provides a summary of the various modes of transmission and dispersal of Moko that have been reported. The removal of excess suckers and the pseudostem of fruited plants produce numerous open wounds through which bacteria can enter the vascular tissues of healthy plants, as well as disperse from diseased plants. Insects incidentally contaminated with the pathogen whilst foraging for pollen/nectar may transmit bacteria to freshly exposed vascular bundles on cushions of abscised flower buds on healthy plants. Insect transmission has also been linked to freshly cut sucker surfaces following pruning (Buddenhagen 1965, 2006; Stover 1972).

According to Buddenhagen (2006) ‘After its establishment in banana Moko disease was moved from area to area by shipment of planting material and later by insects. Insect transmission became increasingly important and today the pathogen is mainly transmitted from flower to flower by insects, leading to the development of epidemics that are driven by the presence of ABB bananas like Bluggoe (Pisang awak). This insect mode of transmission has been confirmed by the isolation of the pathogen from the insects. *Trigona* bees are found to carry most of the inoculum on ABBs in the field’.

With insect transmission, it is proven that bacteria are transmitted from oozing bud to cushions or to female bract scars. It is also possible that bacteria may be transmitted from oozing bud to cut sucker, from fruit to cushion, from cut surface to cut surface and from fruit to cut sucker. Further, it is speculated that transmission from *Heliconia* flower to *Heliconia* or banana flower may be possible. Apart from insects, birds and bats may play a role in transmission (Buddenhagen and Elsasser 1962).

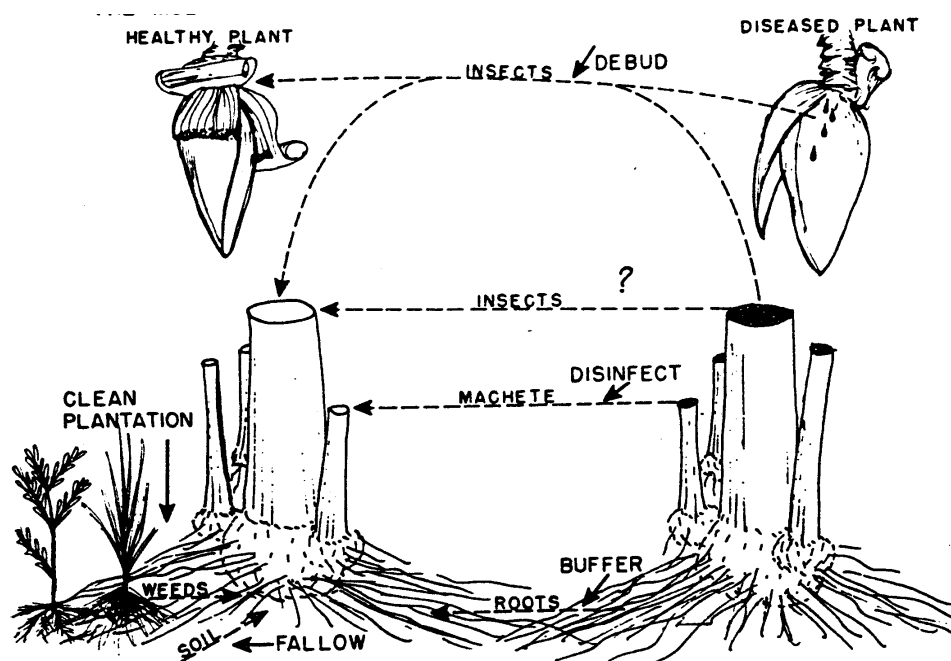


Figure 5.2 Transmission pathways of *R. solanacearum* on *Musa* plants

Source: Stover (1972) – Reproduced with permission from CABI Publishing, CAB International

Table 5.6 Transmission and dispersal mechanisms of *R. solanacearum* race 2

Transmission and dispersal mechanism	References
Mechanical transmission by pruning knives/machetes	Sequeira 1958; Buddenhagen 1960; Buddenhagen and Elsasser 1962; Stover 1972
Insect transmission from diseased to healthy inflorescences	Buddenhagen and Elsasser 1962; Buddenhagen and Kelman 1964; Kenyon et al 1997
Insect transmission from/to open wounds, from diseased to healthy above-ground plant parts other than inflorescences	Stover 1972
Root-to-root transmission (via secondary root emergence)	Kelman and Sequeira 1965; Stover and Espinoza 1992
Opportunity for infection after root wounding during cultural practices	Sequeira 1958; Wardlaw 1972; Lehmann-Danzinger 1987; Hayward 1991
Infection aided by wounds inflicted by nematodes (extrapolated from the synergistic host-nematode relationship in the transmission of *R. solanacearum* race 1 by *Meloidogyne* spp. in tobacco and tomato)	Ashby 1926; Hayward 1991, 2000; Thwaites et al 2000
Movement with infected planting material	Rillo 1979; Buddenhagen 1961, 1986; Black and Delbeke 1991; Fucikovsky and Santos 1992; Stover and Espinoza 1992; Hayward 2000
Movement of contaminated soil (for example, on shoes, animal hooves, farm implements and machinery)	Sequeira 1958; Black and Delbeke 1991; Hayward 1991; Thwaites et al 2000
Rain splash dispersal of Moko bacteria with contaminated soil onto open plant wounds	Sequeira 1958
Dispersal in floodwater or irrigation water	Sequeira and Averre 1961; Fucikovsky and Santos 1992; Coelho Netto and Nutter 2005

Soil transmission

Within the soil profile, Moko bacteria may enter the plant via infection of the roots and corms. Natural root infection by the pathogen occurs through the colonisation of the secondary root axils or the exudation sites at the root tips followed by colonisation of the intercellular spaces and the vascular

parenchyma. Disintegration of cell walls facilitates the further spread of bacteria through the vascular system, which in turn leads to the onset of wilt. Wilting is believed to be caused by blockage of xylem vessels by bacterial cells and polysaccharides produced by the bacteria and subsequent alteration of water movement (Stover 1972; Wardlaw 1972; Genin and Boucher 2002). Bacteria can also enter the corm or roots through wounds created by injuries that expose the vascular bundles (Sequeira 1958; Lehmann-Danzinger 1987), or through root-to-root contact via the point of secondary root emergence (Kelman and Sequeira 1965).

Wardlaw (1972) reported that when the Moko bacterium is poured on or into the soil, it does not survive very long and does not cause wilt disease. Research gained from other field experiments indicated that in order to cause infection of non-wounded *Musa* roots, extremely high numbers of bacteria were required (Kelman and Sequeira 1965). These authors demonstrated that the pathogen could not gain entry directly but was successful in entering the host via points of secondary root emergence when inoculum levels were high ($\geq 5 \times 10^4$ cfu /ml). Sequeira (1958) observed after extensive investigations of the characteristics and mode of dissemination of *R. solanacearum* race 2 that infection of banana plants occurred largely through the above-ground plant parts rather than through the roots. However, 100% infection occurred when roots were wounded (Wardlaw 1972).

Prior to the death of an infected banana plant (100 million to one billion cfu per gram of infected plant tissue), large numbers of Moko bacteria will be released into the soil (Kelman and Sequeira 1965), which greatly increases the inoculum potential. Once bacteria are introduced into the soil, they can spread passively with free water, including flood and irrigation water (Table 5.6). However, the bacterium is believed to be a poor competitor in the soil (Sequeira, 1993; Castañeda–Sànchez et al 2002) or in water that is rich in organic matter (Lehmann-Danzinger 1987). The factors likely to limit the growth and survival of the Moko pathogen are considered in detail in a later section *Survival in the Environment.*

Asymptomatic carrier hosts of *R. solanacearum* race 2 are considered to assist in the survival and subsequent spread of the bacterium to *Musa* spp. as evidenced by the widely accepted practice of suppressing weeds among other measures to achieve control of Moko in commercial banana plantations (Belalcazar et al 1968; Berg 1971; Stover 1972; Jeger et al 1995; Thwaites et al 2000; Granada 2002; Álvarez et al 2008). Belalcazar et al (2004) state that asymptomatic carrier hosts ensure the unlimited survival of the bacterium in a plantation because, unlike susceptible host plants, they are not killed by the pathogen. The presence of suitable hosts in the field is one of the most important factors that prolongs the survival of the bacterium in the soil environment (Sequeira 1962).

Insect transmission

Variations in flower characteristics, such as the sugar content of nectar (Setyobudi and Hermanto 2000), differences in floral structure (Stover and Richardson 1968; Stover 1972), and frequency of male flowering (Denny 2006) influence the transmission pathway of *R. solanacearum* race 2. Insect transmission to bract scars on the inflorescences of Cavendish (AAA genotype) bananas is uncommon in comparison to cooking banana cultivars (Buddenhagen 2006). Insects are attracted to the sweeter nectar of cooking bananas that have *Musa balbisiana* as part of their parentage (Molina 1999; Setyobudi and Hermanto 2000). Infection directly through flowers is not known to occur (Buddenhagen and Kelman 1964), although various erroneous references exist in the literature. It has been suggested that insect transmission via freshly cut sucker surfaces following pruning is possible (Buddenhagen 1965, 2006; Stover 1972), although this observation was not supported by data for any of the banana cultivars. Standard management practices in commercial Cavendish plantations include the removal of the male flower bud after emergence of the last female hand in order to prevent potential insect visitation to male flowers (Stover 1972).

Two to five percent of pollinating insects visiting the flowers and bacterial ooze from the peduncle of infected bluggoe banana plants (*Musa balbisiana* as part of their parentage) can be contaminated with *R. solanacearum* race 2 bacteria (Buddenhagen and Elsasser 1962; Kenyon et al 1997). Of the insects that have been recorded as visiting Moko diseased plants, only bees (*Trigona* spp.), wasps (*Polybia* spp.) and thrips have been confirmed as being surface contaminated with Moko bacteria

(Buddenhagen and Elsasser 1962; Buddenhagen and Kelman 1964; Kenyon et al 1997). Insects are reported to visit the flowers and 'milky white droplets' of the bacterial ooze (containing of $10^8 – 10^{10}$ bacterial cells per gram of infected tissue), at a rate of up to 100 per hour (Buddenhagen and Elsasser (1962). Transmission of the Moko pathogen is rapid when there is high inoculum loads, free availability of moisture, high frequency of insect visits and the moist cushions on the peduncle from which the male flowers have recently abscised are available (Buddenhagen and Elsasser 1962). Transmission to new host plants does not occur if the freshly abscised cushion is not present. Based on 'the frequency counts of insects reaching infectible sites on banana plants (*Musa acuminata* Gros Michel AAA), it was calculated that fewer than one carrier in 100,000 can still account for the present scattered (Moko) disease incidence in plantations in Honduras' (Buddenhagen and Kelman 1964). Even under these disease outbreak conditions of *R. solanacearum* race 2, 'distances of more than 1 mile (1.6 km) that are free of bluggoe act as substantial barriers to dissemination' (Buddenhagen and Elsasser 1962). Bees are the most frequent visitor to banana flowers (Stover 1993), are most frequently contaminated (Kenyon et al 1997) and carry most of the inoculum in the field (Buddenhagen 2006). Further, it has been suggested that the failure to remove large bee nests within 200m of banana plants assists the epidemic spread of the Moko SFR strain (Stover 1993). Transmission of Moko has occurred in isolated instances to bluggoe banana plants as distant as 8 km, though the agent responsible for long distance dispersal is unknown and may be different from insects which disseminate locally (Buddenhagen and Elsasser 1962).

Moko disease is caused by a number of *R. solanacearum* race 2 strains (see Table 1.2; Denny 2006). The SFR and A strains of *R. solanacearum* race 2 are closely associated with insect vectors and the rapid spread of Moko disease in Honduras (Buddenhagen and Elsasser 1962; Buddenhagen and Kelman 1964) and other countries in Central and South America (Denny 2006). In Cavendish bananas, the B strain (that occurs in the Philippines) is mainly soil transmitted and infrequently transmitted by insects because infected plants exude relatively little bacterial ooze (Denny 2006). The B strain is not associated with the rapid dispersal of Moko by insects in Central America (Buddenhagen and Kelman 1964).

The transmission of *R. solanacearum* race 2 from symptomatic banana fruit to host plants was considered an option for the transmission of *R. solanacearum* in the disease outbreak in Honduras but this has not been proved (Buddenhagen and Kelman 1964). It was believed possible that Moko bacteria become available for insect vectors via cracks in visibly diseased fruit in banana plantations (Buddenhagen and Kelman 1964). It is likely that specialist pollinating insects, shown to vector *R. solanacearum*, will not be attracted to isolated banana waste (as it is not a nectar, pollen, propolis or abundant water source) when normal floral resources are available. Further, any resources (residual moisture and sugars from decaying fruit) that may attract specialist pollinating insects to banana waste are transient, as desiccation and microbe mediated decay (see section below – *Survival in banana waste*) will remove resources attractive to pollinators in a very short period.

It is more likely that invertebrate groups associated with waste plant material, particularly the complex polysaccharides that can take many weeks to decompose, will have the life-history and opportunity to visit banana waste. Detritivores constitute the majority of the invertebrate biomass pyramid in most environments and provide a key role in organic matter turnover (Paoletti et al 2007). Detritivores are diverse in taxa and include isopods, amphipods, millipedes, oribatid mites, dipteran larvae, earthworms and collembolans. Detritivore faecal material enhances soil structure and is a preferred substrate for microbial colonisation, thereby indirectly accelerating the decomposition of organic matter. Detritivores also disseminate fungal spores locally and their presence can moderate the effects of plant pathogens (Paoletti et al 2007).

Oribatida mites are considered to be the most important detritivores in the decomposition process and are found in all Australian terrestrial habitats with some locations supporting more than 90 species and 100 000 mites m^{-2} (Paoletti et al 2007). They influence decomposition rates directly by shredding organic matter as they feed on it and indirectly by producing faecal pellets which enhance the activity of bacterial and fungal colonisers that metabolise organic matter (Paoletti et al 2007). Berthet (1964) (cited in Lilleskov and Bruns 2005) estimated movement of 20.5 cm/day for oribatid mites, although much lower rates (≈20 cm/week) have been reported for both mites and edaphic springtails. Given the

small dispersal distances and terrestrial habit of oribatida mites (and detritivores in general) they are not considered vectors of Moko.

Other functional groups likely to visit banana waste are foragers and include the numerically abundant cockroaches and ants (CSIRO 1991). Of the cockroaches in Australia, those species most relevant to banana waste are likely to be the German and American cockroaches that are abundant and important pests (CSIRO 1991). The juvenile stages of cockroaches do not have wings (CSIRO 1991) and although the adult German cockroach (*Blattella germanica*) is winged, it is not known to fly (Benson and Zungoli 2001) and forages by walking (Cloarec and Rivault 1991). The American cockroach (*Periplaneta americana*) is primarily a ground dweller but will fly short distances under certain conditions (such as warm nights, Benson and Zungoli 2001; Epestsupply 2008) although it does not have the required physiology for prolonged flight (Chino et al 1992). These cockroaches generally exhibit trail-following behaviour related to faecal chemical cues (Miller and Koehler 2000) or aggregation behaviour in harbourage sites (Leoncini and Rivault 2005). Their forage trails lead from sites of harbourage to regular sources of food and water. Male German cockroaches were observed making at least 1 foraging trip per day, 7 days out of 10. Gravid females were shown to forage only 3 days out of 10, rarely leaving their harbourage, while females and nymphs fell between males and gravid females in the number of foraging excursions made in search of food or water (DeMark 1992 as reported in Miller and Koehler 2000). In American cockroaches, non pregnant females are the most active foragers and can cover more than 10 m in a night (Schal et al 1984). The German cockroach is a general feeder, but is particularly attracted to fermented foods and beverage residues such as beer spills (Epestsupply 2008). The American cockroach is normally found outside and preferentially feeds on decaying organic matter. As foraging trails largely determine the search patterns in cockroaches and foraging events are relatively infrequent, the banana waste would need to be discarded in close proximity to established harbourage sites and foraging trails for it to be encountered readily.

Ants are considered one of the most ecologically important and prominent animal groups in Australia (CSIRO 1991). Green tree ants are a dominant species in sub tropical and tropical regions (Bluthgen and Fielder 2002; Bluthgen and Stock 2007) where Moko hosts are most likely to grow. Green tree ants are territorial and a colony typically covers an area of 170 m^2 containing several trees (Peng et al 1998). They are primarily arboreal but workers do forage on the ground and can only move food resources to the arboreal nest by walking. For terrestrial species, the range over which they forage can be restricted by discrete territories. *Iridomyrmex purpureus* is a widely distributed and dominant species in Australia (Greenslade 1987), where ant colonies can have territories that cover as little as 15 m^2 (van Wilgenburg 2007).

Given the primarily flightless habit and restricted territorial range of the foragers that are likely to visit banana waste, any inoculum that adheres to these insect groups will be lost quickly due to their frequent contact with the substrate they walk across.

In contrast to the detritivores and foragers, insects that use flight as the primary mode of movement are most likely to vector Moko inoculum to suitable host plants. The diverse and numerically abundant insect families of flies and beetles are commonly associated with plant products (CSIRO 1991). In tropical and sub–tropical Australia, drosophilids are an important and diverse insect group associated with fallen fruit of many plant species across a variety of vegetation types (van Klinken and Walter 2001) and adults are attracted to macerated whole banana fruit (van Klinken et al 2002). Most species of Drosophila in Australia are fungivorous with many eating yeasts growing on decaying fruits (CSIRO 1991). For example, *Drosophila melanogaster* preferentially selects food resources high in fermented products (Zhu et al 2003) and low in sugars (Yang et al. 2008). Field studies have shown that in the presence of food, *D. melanogaster* moves over only very short distances of 1–7 metres per 24 hours (McKenzie 1974; Lefranc et al 2001). When food deprived, *Drosophila* are able to move over much larger distances (Coyne et al 1987).

Species of the fly family Muscidae can be associated with banana plantations in Australia. *Atherigona orientalis* has been recorded to be associated with a range of rotting fruits and occasionally *Musa* spp (Pont 1992), and *A. orientalis* is regulated under New Zealand import conditions for fresh banana fruit from Australia (Biosecurity New Zealand MAF, Wellington, New Zealand 2006). *Atherigonia*

orientalis is typically found in damaged plant material, including flowers, fruit, growing points, stem, leaves and fruit, but it can also develop in dung and dried insects (Pont 1992; Pont and Magpayo 1995). However, it is often very difficult, where infestation is found, to determine if the fly caused the initial damage, or had infested an already damaged plant. For example, Al-Janabi et al (1983) found that in *Brassica oleracoa* attacked by other insects, caused a soft rot, which then attracted *A. orientalis*. Cahill (1992) noted in his studies that sweet peppers (*Capsicum annum*) cultivars attacked had an open calyx and adjacent groves and similarly rock melons attacked were ones, which had slipped or separated from the vine and so had a broken calyx. These studies suggest the potential for adult flies to become contaminated with Moko bacteria from banana waste and transfer them to banana plants at a stage favourable for infection and colonization by the bacteria.

Within the beetle family, many species of Carpophilus beetles are serious pests of a large range of fruits in Australia (CSIRO 1991, James et al 1994), are attracted to banana fruit (Phelan and Lin 1991) and are known to be associated with fresh wounds on plants (Jurwik et al 2004). In Australia, Carpophilus beetles are attracted by fermenting materials for nutrition and reproduction (James et al 1994) and will therefore be attracted preferentially to any fruit that is decaying (Phelan and Lin 1991). Carpophilus beetles have been shown to be closely associated with plant pathogens and act as the primary vector in a co-evolved system. In this system, beetles are attracted to the sporulating fungal mat of the pathogen by aromatic volatiles where they feed, mate and oviposit and then infect host plants by visiting wound sites (Jurswik et al 2004). Seventy-five percent of beetles visiting plant wound sites were found to be contaminated with inoculum, with up to 18 000 cfu per beetle (Jurswik et al 2004). Under these types of conditions, when beetles had access to wounded plants, transmission success of the plant pathogen varied from 3–29% (Camilli et al 2007). The distance Carpophilus beetles can disperse is large and they may potentially vector disease large distances. Yet, in the example of two Carpophilus beetles that vector *Aspergillus flavus*, an important disease on maize, inoculum levels on the beetles typically declined to zero at 14 m from an inoculum source of sporulating colonies of the pathogen (Olanya et al 1997).

The transmission success of a vectored plant pathogen can be influenced by the interplant distance in a natural population of the diseased host plant (Bieres and Honders 1998). Infection probability strongly increased with the frequency of diseased hosts, where as the number of diseased hosts had no effect. The probability of infection increased more strongly with increasing disease frequency where interplant distances were small. This supports the theory that transmission should shift from a more frequency-dependent to a more density-dependent process when host search (interplant) time increases relative to handling time (host contact period). Knops et al (1999) also showed that a reduction in the plant richness of an area enhances the spread of plant fungal disease by effectively increasing the density of the host plant and reducing the richness of the insect assemblage. Therefore, the random disposal of uncontrolled banana waste infected with Moko bacteria will limit the frequency that vectors will come into contact with inoculum, limiting the vectors ability to accidentally transmit a pathogen.

The diversity of invertebrates likely to visit banana waste in Australia is likely to be great. Of these species only a small proportion will have the potential to vector inoculum to a suitable host plant. Successful transmission by a vector depends on several coexisting conditions, including (i) the availability of viable inoculum (ii) the presence of active vectors to transmit the pathogen (iii) a fresh, xylem-exposing wound receptive to infection by the pathogenic inoculum present on the vectors (iv) the coexistence of these events during temperature conditions favourable to Moko and (v) the absence of microbial deterrents to infection at or introduced to the wound surface (see *Survival in the Environment*). The vectors most likely to visit banana waste are preferentially attracted to decaying and fermenting tissue, decreasing the chances of vectors finding freshly disposed waste. Additionally, decomposers and detritivores impact greatly on the persistence of pathogens in the environment and foragers are likely to dilute the inoculum across unfavourable substrates. Therefore, the likelihood of insect vectors in the field picking up any Moko bacteria originally on a piece of banana waste is rapidly reduced through time by the action of a diverse fauna interacting with a range of saprophytes (see *Survival in banana waste*). When inoculum successfully adheres to a vector, although insect

dispersal distances can be large, insect flight behaviour and inoculum persistence on the vector will influence the distance over which successful transmission will occur.

Other modes of transmission

Plant wounds produced by nematodes do not seem to have the same importance for infection by *R. solanacearum* race 2 (Lehmann-Danzinger 1987). Synergistic interactions of nematodes with other races of *R. solanacearum* are well documented and thought to be important (Hayward 1991, 2000). Banana and plantain plants neighbouring Moko-infected plants often remain healthy in spite of the presence of nematodes (Lehmann-Danzinger 1987). It could be that the saprophytic bacteria and fungi present in decaying roots which accompany nematode attack prevent the bacterium from reaching the xylem vessels (Lehmann-Danzinger 1987).

Dispersal of *R. solanacearum* race 2 by rain splash from soil may occur (Sequeira 1958). Although there is no information on splash dispersal of *R. solanacearum* race 2, it is thought unlikely to be significant in comparison to insect transmission. Epiphytic populations of *R. solanacearum* have not been described and they are not known to play any role in the epidemiology of Moko disease. There is no evidence of the dissemination of *R. solanacearum* race 2 in aerosols as reported from other bacterial plant pathogens such as *Erwinia amylovora*, fire blight of apples and pears, and *Pseudomonas syringae* pv. *syringae*, blossom blight (Goto 1990).

However, there is ample evidence of short- as well as long-distance spread of *R. solanacearum* race 2 through movement of infected propagation material (refer to Table 5.6). Local and international dispersal of the *R. solanacearum* race 2 is commonly associated with the movement of planting material, such as the movement of banana corms from Central America to the Caribbean and to the Philippines, and on *Heliconia* rhizomes within Central America and the Caribbean, and from Hawaii to Australia and India (Phelps 1987; Hayward 2000).

There appears to be no published information concerning the survival of *R. solanacearum* race 2 on the surface of green unripe or ripening fruit under natural conditions as it is known to occur under experimental conditions (Soguilon 2003b). Internal infection of fruit occurs through the vascular system from the rhizome upwards through the pseudostem or, in the case of insect transmission to the inflorescence, downwards through the pedicels and the peduncle into the pseudostem. The pathogen can be readily isolated from the vascular system of the peduncle and the pseudostem of symptomatic plants, and has recently been isolated from asymptomatic fruit of Cavendish banana cultivars that exhibited vascular browning in the peduncle (Soguilon 2003a). Published records on the introduction and establishment of *R. solanacearum* race 2 as a result of marketing infected Cavendish banana fruit were not found.

Survival in the environment

Ralstonia solanacearum has been reported to survive for 2–11 days in films dried to glass surfaces as reviewed by Kelman (1953). Smith (1914) reported on the survival of bacteria on cover slips wetted in a nutrient liquid culture and placed in covered sterile petri dishes and left at room temperature in the dark that would limit the evaporation of moisture from the cover slip. Even under these favourable moisture conditions, greater than 75% of bacteria were dead after 5 days and all by 6–9 days (Smith 1914). The work by Sequeira (1958) involved glass rods covered in a nutrient liquid culture that was drained and the rods (from 12–16) were then placed into petri dishes with lids and under these conditions bacteria survived for 11 days. In contrast, when glass rods were immersed in a bacterial suspension and then arranged so evaporation could occur under laboratory conditions, most bacteria were dead by 24 hours and no bacteria survived for 48 hours (Moraes 1947 as reported by Kelman 1953). In diseased plants, when pieces of banana sheath were removed and maintained under moist conditions, no Moko bacteria could be isolated after 8 days (Wardlaw 1972). Under high humidity conditions, *R. solanacearum* has been shown to survive *in vitro* and *in vivo* between 6–11 days and when evaporation is allowed for only 24–48 hours. In comparison to other non-spore forming plant pathogenic bacteria the survival of *R. solanacearum* is remarkably short when subjected to desiccation

(For example, *Xanthomonas begoniae* can survive for 75 days at 25 °C; Dowson 1949; Sequeira 1958).

Under field conditions, the survival of *R. solanacearum* race 2 depends on a warm, moist, dark and preferably sheltered environment within plants, as the pathogen is highly sensitive to desiccation (drying out), light, and high and low temperatures (Kelman 1953; Jeger et al 1995; Momol et al 2005; Swanson et al 2005). The bacterium does not produce desiccation-resistant resting cells and lacks carotenoid pigments that protect cells from the deleterious effects of UV radiation (Kelman 1953; Sequeira 1962, 1993). *Ralstonia solanacearum* race 2 is also highly susceptible to antagonistic microbiota and organically rich environments (Buddenhagen 1965; Lehmann-Danzinger 1987; Roy et al 1999; Sequeira 1993).

Survival in soil

In the soil environment, large population densities of *R. solanacearum* race 2 derived from dying plants can survive for up to two years (defined as short-term) where their survival is intrinsically linked with the colonisation of roots of symptomatic and asymptomatic hosts and non-hosts as well as the nutrient-rich rhizosphere of roots of plants in general (Granada and Sequeira 1983). Survival for more than two years (defined as long-term) depends on the ability to infect roots of suitable hosts, as research has shown that all populations of *R. solanacearum* in the presence of non-hosts declined over time (Granada and Sequeira 1983). Research on the related pathogen *R. solanacearum* race 3/biovar 2 infecting geraniums suggests that *R. solanacearum* populations derive primarily from the infected plant and not from residual inoculum within the soil (Swanson et al 2005). Exopolysaccharides enveloping *R. solanacearum* race 2 cell aggregates oozing from infected plant material may also aid in the survival of bacterial cells due to prevention of water loss (Allison 1998), one of the primary causes of cell death (Kelman and Sequeira 1965).

It has been theorised that *R. solanacearum* may not be a strong competitor in the soil (Sequeira 1993; Castañeda–Sànchez et al 2002), as the long-term survival of the pathogen is associated with infection of living plant material but not in the presence of soil *per se* (Granada and Sequeira 1983; Thwaites et al 2000; Swanson et al 2005). Survival of *R. solanacearum* race 2 in the soil environment is most influenced by high and low temperatures and low moisture availability (Sequeira and Averre 1961; Kelman and Sequeira 1965; Wardlaw 1972; Fucikovsky and Santos 1992; Pereira and Normando 1993). The presence of live plant parts in deeper soil layers has also been found to enhance the survival of *R. solanacearum* biovar 2/race 3 potato brown rot cells, presumably due to lower predation by antagonistic micro-organisms and lower temperature fluctuation (van Elsas et al 2000). Species belonging to the *R. solanacearum* complex are sensitive to extreme conditions including dry conditions (Kelman 1953), frost (Kelman 1953; OEPP/EPPO 2004; Momol et al 2005), salinity (Kelman 1953), presence of metal ions in the soil (Grey and Steck 2001) and pH (that is, no growth lower than pH 4 and higher than pH 9) (Michel and Mew 1998). *Ralstonia solanacearum* does not occur in calcareous soils with high pH that promote proliferation of antagonistic mirco-organisms (Momol et al 2005). Extreme conditions led to the accelerated decay of *R. solanacearum* in soil (Hayward 1991). Freezing and the formation of ice crystals in soils, and soils with low water holding capacity/field capacity (10–15% soil moisture level) for instance led to a reduction of populations of potato brown rot (*R. solanacearum* race 3/biovar 2) which dropped below detectable levels within 15 days (van Elsas et al 2000). Although there are no similar data available for soilborne *R. solanacearum* race 2, it is expected to behave similarly.

Survival in water

Axenic cultures of virulent *R. solanacearum* race 2 bacteria are known to survive for many years in sterile distilled water at 20–25 °C, which is regarded as an acceptable means of long-term storage (Buddenhagen 1994; van Elsas et al 2001). However, the bacterium is believed to be a poor competitor in organically enriched water (Lehman-Danzinger 1987). This is most likely due to the presence of antagonistic micro-organisms, such as grazing and parasitic protozoa.

Evidence from the literature indicates that floodwater is likely to be an adverse environment for Moko bacteria free in suspension as it is for other soilborne plant pathogens. Two mechanisms have been proposed, one functional in the short term the other in the long term. In the latter case, bodies of stagnant water enriched with organic matter become anaerobic and favourable for the fermentative activity of a variety of anaerobic bacteria. Momma et al. (2006) showed that under these conditions acetic and butyric acids were produced that were suppressive to both Fusarium wilt and bacterial wilt (*Ralstonia solanacearum*). These authors propose the term Biological Soil Disinfestation (BSD) for this process. BSD is likely to be part of the basis for the efficacy of flood fallowing for the control of bacterial wilt as reported in much earlier literature. In the early decades of the 20th century, Dutch scientists in Indonesia showed that flood fallowing of soil, after vegetable crops susceptible to bacterial wilt, led to a sharp decline in soil populations of the bacterial wilt pathogen. This early work was reviewed by Kelman (1953). In reference to bacterial wilt in Indonesia Thung (1947) states that, "Wet rice cultivation has been observed to favour the elimination of soil infestation". In Nepal, irrigated rice was shown to be a suitable break crop in bacterial wilt infested areas (Pradhanang and Momol 2001). These authors showed that the population of *R. solanacearum* in soil declined to an undetectable level three months after the rice harvest.

The BSD phenomenon described in the previous paragraph is unlikely to be a major factor affecting the survival of the Moko pathogen in the flood plain of the Tully Valley, North Queensland, for example, except in bodies of stagnant water.

Recent work shows that lysis by bacterial viruses (bacteriophages), predation by protozoa, and the activity of other antagonistic micro-organisms contribute to the reduced persistence of *R. solanacearum* in river water. Álvarez et al. (2007) investigated the influence of native microbiota on the survival of the potato strain of *R. solanacearum* in river water microcosms in Spain at 14 or 24 °C for a month. At both temperatures the densities of the bacterial pathogen remained constant at the initial levels in control microcosms of sterile river water while, by contrast, declines in the populations of the introduced strain were observed in the non-sterile microcosms. These decreases were less marked at 14 °C. Lytic bacteriophages present in the river water were involved in the most immediate effect on the decline of pathogen populations, within days of exposure, but indigenous protozoa and bacteria also contributed to the reduced persistence in water. In contrast to river water, turbulent floodwater would be expected to contain surface soil in suspension as well as plant debris and other organic material.

In infected banana plantations where inoculum levels of Moko will be very high, survival in floodwaters can occur. In Brazil Moko disease of banana was more prevalent in plantations subject to periodic flooding by river water than in those not flooded. Moko disease occurred in 30 of 52 (57.7%) plantations subject to periodic flooding, but in only 1 of 55 (1.8%) of plantations not subject to flooding (Coelho Netto and Nutter 2005). The role of floodwater in the survival and spread of an established disease where high levels of inoculum is leached directly into the soil contrasts, in several aspects, with the introduction of the Moko pathogen in banana waste (see *Survival in banana waste*).

In summary, the initial inoculum level, biotic and abiotic factors will affect the survival of the Moko pathogen suspended in river or in floodwater. The adverse effect is most likely due to biological factors in the short term and to abiotic factors such as the BSD effect in the long term.

Non-culturable state of *Ralstonia solanacearum*

Ralstonia solanacearum race 2 may enter into a viable but not culturable (VBNC) state. This is known to occur for the related pathogen *R. solanacearum* biovar 2/race 3 that causes potato brown rot. *Ralstonia solanacearum* biovar 2/race 3 enters into a viable and metabolically active, but non-culturable state when exposed to temperatures of 4 ºC and below (van Elsas et al 2000, 2001, 2005; van Overbeek et al 2004), in the presence of copper ions (Grey and Steck 2001), or when starved of nutrients (van Elsas et al 2001). VBNC cells are undetectable by standard bacterial culturing methodology but are detectable by fluorescent microscopy (van Elsas et al 2000).

It has been suggested that the VBNC stage constitutes a type of resting or survival form of non-differentiating bacteria, including Gram negative bacteria such as *Ralstonia* spp. (van Elsas et al

2000), although this has not yet been confirmed. Various theories on the VBNC stage have been considered, such as cells being alive with basal metabolic activity, dormant, dying, non-viable or resuscitable to a culturable stage without the loss of virulence (van Overbeek et al 2004). It is not known if *R. solanacearum* race 2 would enter into a VBNC state and neither is it known if the pathogen would enter the VBNC state in or on banana tissue as the VBNC phenomenon is not yet completely understood.

Effects of temperature on the growth of *R. solanacearum* race 2

The temperatures relevant to the importation of banana fruit include those that are present in the Philippines and Australian environments (Figure 2.1 and Figure 2.2 respectively), during transport (at 13–14°C for 10–14 days) and ripening (at 14.5–21°C). The growth of *R. solanacearum* considered in this and following sections refers to the multiplication of bacterial cells or population growth of the pathogen.

There is no published data on the temperature relations of the Moko bacterium. However, strains affecting other hosts (eg race 3 that affects potatoes) indicate that the optimum temperature range for Moko bacteria growth under *in vitro* conditions is 32–35 °C, the minimum at 8–10°C, and the maximum at ~40 °C (Kelman 1953). Gram-negative bacteria similar to *R. solanacearum* may have a generation time in the range of 45-60 minutes under optimum conditions *in vitro*. There is no data on doubling time in a susceptible plant under optimum conditions. An estimate of two hours or more is assumed for *R. solanacearum* race 2.

The effects of temperature on the relative growth rate of *R. solanacearum* race 2 are presented in Figure 5.3. These data were extrapolated from *in vitro* studies with other strains of *R. solanacearum* (Kelman 1953) using a model in the form of $R=\exp[-((T-T_{opt})/(T_{opt}-T_{range}))^2]$, where R is the growth rate at temperature T relative to that at the optimum temperature ($T_{opt} = 33.5$ºC) and T_{range} is the temperature at which the growth rate is 1/e (approximately 37%) of that at T_{opt}. The T_{range} is assumed to be 10 °C. This model is similar in form to that used for black Sigatoka (Part C, Appendix 6) and is derived from Allen et al (1992). This model does not make accurate predictions at temperatures greater than T_{opt} but such temperatures are not particularly relevant to this risk assessment.

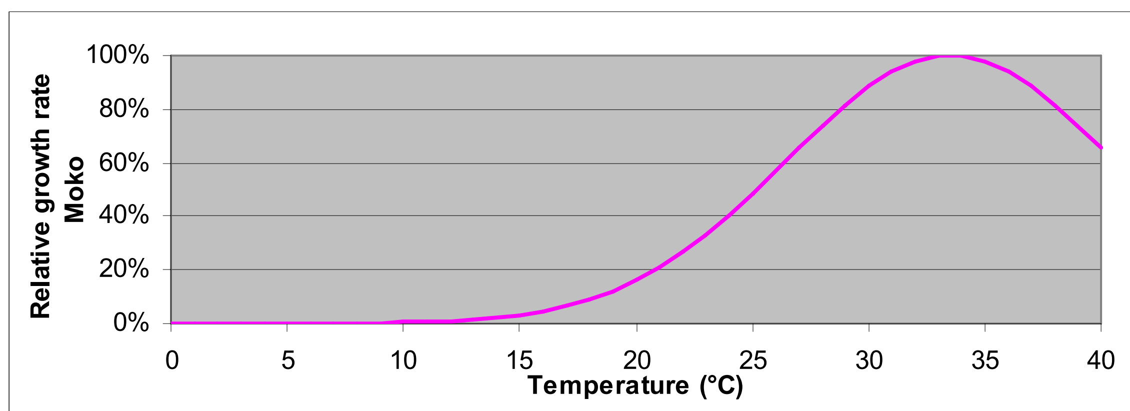


Figure 5.3 Effects of temperature on the relative growth rate of *R. solanacearum* race 2

Source: derived from Allen et al (1992)

It is estimated that if the doubling time at the optimum temperature is 45–60 minutes, the doubling time at 25 °C is 92–122 minutes and growth effectively ceases below 10 °C as observed by Kelman (1953).

The average monthly temperatures (the mean of average daily minimum and maximum temperatures) for selected locations in the Philippines and Australia are presented in Figure 2.1 and Figure 2.2 respectively. The estimated relative growth rates for these selected locations are presented in Figure 5.4

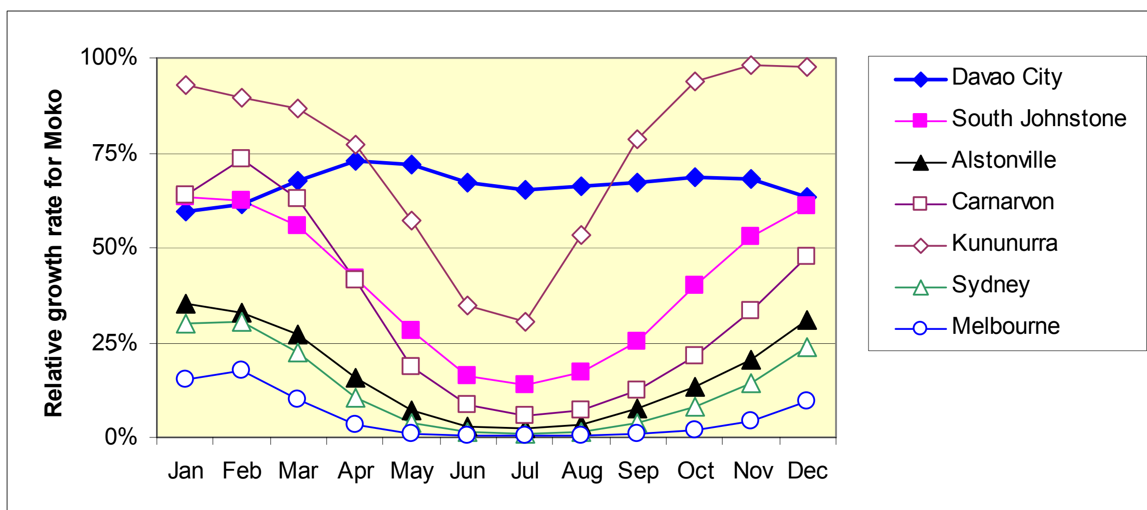


Figure 5.4 Predicted monthly relative growth rates of *R. solanacearum* race 2
Source: derived from Allen et al (1992)

Figure 5.4 shows the effect of location on the relative growth rate of *R. solanacearum* race 2 without taking into consideration the possible overriding effect of water availability on growth. The estimates in Figure 5.4 indicate that the relative growth rates of *R. solanacearum* race 2 at temperatures typical of Davao City in the Philippines, and of the summer months around South Johnstone and Carnarvon, Australia, are approximately 50% of those at the optimum temperature. If other environmental conditions, particularly water availability are conducive, some growth of *R. solanacearum* race 2 could be expected in most areas of Australia in the summer months, but growth in subtropical and temperate areas of Australia would be greatly restricted by cold temperatures in the winter months. It is also evident that the average temperature is near optimal for *R. solanacearum* race 2 during the summer months at Kununurra, Western Australia.

Growth and survival of *R. solanacearum* race 2 in banana fruit

At harvest and packing, the concentration of bacteria present in asymptomatic fruit is likely to be from very low numbers of cells to 10^8 bacterial cfu/gram of tissue, and on average would not exceed 10^6 bacterial cells/gram of tissue. Independent advice from C Hayward (Bacteriologist, consultant, pers comm 20 September 2006) supported these values. When bunches are processed in the dip tank, the potential exists for bacteria from asymptomatically infected fruit to contaminate clusters free of Moko bacteria. When banana tissue is cut, there is a rapid exudation of banana latex (Kallarackal et al 1990) and a smaller amount of other plant fluids (David Turner, Associate Professor, Plant physiologist consultant, pers comm., 10 June 2008) into the dip tank water. The ABGC (2007) quote unreferenced work from the Queensland Department of Primary Industry and Fisheries that a total of 130mm of sap exude from a banana bunch which could be higher under more favourable conditions. Contaminated pruning tools will also transfer Moko bacteria to the water and freshly cut surfaces of banana tissue directly. Since Moko bacteria do not reside in the laticifers, the amount of fluid exuded from the xylem (and containing bacteria) will be small compared to the volume of latex. The latex exuded from the laticifer is replaced by fluid from surrounding tissue (Kallarackal et al 1990) and as this occurs, dip tank water (containing bacteria) will be drawn into the tissue of banana fruit, including the xylem. This has been shown experimentally with suspensions of fungal spores that can enter the xylem of freshly cut banana fruit (Greene and Goos 1963). During exposure to the dip tank water the bacteria will be exposed to a range of conditions different to the host tissue within which they normally reside. The dip tank water will be cooler than ambient air temperature but still suitable for Moko survival. The nutritional status of the water is likely to be low, but the bacteria will have endogenous energy reserves which can be utilised in times of carbon deficiency (Denny 2006). In the absence of any sanitation of the dip tank water, the growth of Moko bacteria will be retarded but survival is unlikely to be adversely affected. Viable bacteria will infect new host material. Moko bacteria that infest the

surface of the fruit is unlikely to survive for any length of time as it will be exposed to a range of extreme environmental conditions as discussed below.

Several factors affect the growth and survival of *R. solanacearum* race 2 in fruit after harvest. The most important of these are temperature (as described above) and the availability of water for the bacteria to survive.

In plants and the soil matrix, the availability of water is usually measured as water potential. Water potential is described by a scale from zero, for pure water, to increasingly negative numbers for water containing dissolved substances. For example, sea water has a water potential of –1.2 MPa and when the soil is dry enough to cause plants to wilt its water potential is typically –1.5 MPa. Water potential is the potential energy of water relative to pure water under standard conditions. It is a measure of the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or matrix effects including surface tension. Water potential is measured in units of pressure and is commonly represented by the Greek letter Psi (Ψ) and is a concept that is useful in understanding water movement in plants and in soil. Water potential is considered to be a concept applicable to fresh green bananas from harvest, transport, storage, ripening to distribution and marketing. Table 5.7 shows the treatment of bananas during this sequence of events.

Table 5.7 Sequence of changes in the environment to which fresh green bananas are exposed from harvest to retail

Sequence of Events	Duration (days)	Temperature (°C)	Relative Humidity (%)	Water Loss
Shipping Philippines to Australia	10–14	13–14	High (95)	Minimal
Storage prior to ripening	2–5	13–14	High (95)	Minimal
Ripening of fruit under controlled conditions	1–2	14.5–21	Moderate (70–80)	Increasing
Distribution and marketing	2	Variable with location 10–30	Low to Moderate (40–80)*	Increasing
Retail to waste disposal	1–10	Variable with location 10–30	Low to moderate (40–80)*	Increasing

* Based on data from the Australian Bureau of Meteorology on the annual range of atmospheric relative humidity across the northern, eastern, southern, and south west coast of Australia. Major urban centres in each state and territory are represented. Within refrigerated air-conditioned premises the relative humidity is likely to be lower.

The interval between harvest and disposal as waste is generally between 16–33 days. Evidence concerning the growth and survival of the Moko pathogen between harvest and disposal is considered below.

Prior to ripening, fresh green bananas are held at 13–14°C, a temperature that is close to the minimum temperature for growth (see *Effect of temperature on the growth of R. solanacearum race 2*). The generation time of the Moko pathogen is greatly extended and the relative growth rate is a very low percentage of the maximum rate of growth as shown in Figure 5.4. It is likely that loss of water after harvest is an important factor affecting growth of the Moko pathogen.

Although water content and water potential are not equivalent, water loss will lead to a reduction in water potential. Small changes in water solute concentration such as from water loss will result in large changes in water potential (see figure 3-6 p54, Salisbury and Ross 1992). From the moment of harvest all arable and horticultural products lose weight. These weight losses consist of carbon dioxide loss during respiration and water loss by evaporation of water from the product. The extent of water loss depends on the vapour pressure difference between the product and the surrounding air. The smaller this difference the lower the rate of water loss (Scheer 1994). The water loss from bananas during transport and storage in plastic film, and the reduction in water potential, are likely to be minimal.

There have been many studies confirming the weight loss of cooking and dessert bananas after harvest, during storage and the ripening process ranging up to 15% depending on temperature and relative humidity (Ahmed et al 2001; Ahmed et al 2006a & b; Akkaravessapong et al 1992; Burdon et al. 1993; Ratule et al. 2006; Saengpook et al. 2007). Even under high relative humidity (90–95%) at low temperatures (16 °C) ripening bananas lost 1.1% in weight over 11 days (Ahmad et al 2006a).

Further work has confirmed water loss directly from the peel as measured at the pedicel (Imsabai et al 2006; Saengpook et al 2007). The physical basis for water loss even under high humidity is explained by the capacity of the air for water vapour where small decreases in relative humidity result in large decreases (on a logarithmic scale) in the water potential of air (Salisbury and Ross 1992). Burdon et al (1993) found that the epicuticular wax at the surface of banana fruit was a more important determinant of water loss than stomatal length or density. There is a natural degradation of the epidermal cells and loss of cuticle wax in Cavendish bananas that increases through time post harvest (Williams et al 1989). The loss of surface integrity of banana peel will increase the avenues for water loss from fruit.

In the only study found on water loss from bananas that had reached a stage suitable for retail sale, the water content of the pedicel from whole fruit decreased by 32% over nine days at 25 °C and 68% relative humidity (Saengpook et al 2007). The conditions in this experiment are comparable to those bananas would be exposed to at retail outlets and in households. The water loss in this experiment was measured at the pedicel, an area of lignified tissue compared with the rest of the peel (Imsabai et al 2006). The rate of water loss in this experiment increased through time and is unlikely to slow in dry environments post–human consumption, when the integrity of the peel is further damaged.

During ripening of banana fruit there is a marked increase in the rate of respiration and physiological changes occur that affect the osmotic pressure and water potential of the pulp. The conversion of starch to sugar increases solute concentration in the pulp and leads to a decrease in osmotic potential and an increase in the turgor pressure (Fukushima et al. 1980). The osmotic potential of the peel also decreases during ripening but to a much lesser extent than in the pulp (Stratton and von Loesecke 1931). The osmotic gradient between peel and pulp is such that there could be a net water loss from the peel to the pulp until equilibrium is reached. However, Turner (1997) has pointed out that water movement to the pulp may not occur as this movement is also dependant on the turgor pressure of the tissue that, in conjunction with water potential, will govern the direction of water movement. Further there is evidence that at the junction of the peel and the pulp there is a barrier to oxygen diffusion (Perez and Beaudry 1998; Turner and Fortescue *in press*) that may restrict water flow between the tissues. In contrast to the water potential conditions in banana fruit described above (–0.62 MPa to –2.91 MPa), Moko bacteria are typically vascular pathogens of live banana plants, that even under severe water deficits maintain a water potential in the leaves of approximately –0.35 MPa or above (Kallarackal et al 1990). In addition to water loss during commercial ripening, transpiration of water via the peel and pedicel continues through to retail sale (Saengpook et al 2007) that may decrease water potential further, depending on the balance between the osmotic potential, the volume of the cells and the turgor pressure.

There are a lack of data on the direct effect of decreasing water potential in the banana fruit on the growth and survival of Moko bacteria in ripening banana tissue. An estimate can be obtained from the effects of water potential in soil on the growth and survival of Moko bacteria (Akiew 1986; Nesmith and Jenkins 1895). Nesmith and Jenkins (1985) have recorded the growth and survival of *R. solanacearum* race 1 in a range of soil types that have been sterilised or not. To confirm the suitability of soil as a medium for *R. solanacearu*m race 1, bacteria grown in culture grew equally well with or without leachates from all soils tested. The conducive nature of the soils to freshly cultured bacteria of *R. solanacearum* race 1 was shown where the bacterial population increased from 5 X 10^5 to 1 X 10^7 cfu/ gm soil over one to two weeks in all soil types tested if the water potential is suitable (–0.05 to –0.1 MPa), similar to that which occurs in banana xylem. The data from Nesmith and Jenkins (1985) indicate that sterile soil is not an adverse environment for Moko bacteria though limited by a lack of suitable nutrients and where water potential, is the dominant factor affecting growth and survival. Under decreasing water potentials the growth of bacteria slows and then decreases to death (Nesmith and Jenkins 1985; Akiew 1986). In using the *R. solanacearum* race 1 soil survival data to estimate Moko survival in banana tissue it is important to recognise physical differences between soil and plant tissue. The Nesmith and Jenkins (1985) data show under favourable water potentials that rapid growth of *R. solanacearum* can occur. However, in soils with high clay content, water gain/loss can cause significant swelling and shrinking that may physically affect the survival of bacteria. The soils used by Nesmith and Jenkins (1985) have a low potential for swelling or shrinking (NRCS 2008). Further, the clay content of the soils used in the experiments did vary and yet no difference in the survival of *R.*

solanacearum race 1 occurred between sterile soil types at the lowest water potential. Nesmith and Jenkins (1985) showed that *R. solanacaearum* race 1 could survive for two weeks in 3 out of 4 soils that were too dry for plants to extract water from them. In un-sterilised soil, 2 of the four soils were 'suppressive' and contained organisms unfavourable for the growth and survival of bacteria.

Using data from Nesmith and Jenkins (1985) and the water potentials recorded for intact ripening banana fruit (Stratton and von Loesecke 1931; Fukushima et al. 1980), a model can be constructed to determine the likely growth of bacteria (David Turner, Associate Professor, Plant physiologist consultant, pers comm., 25 June 2008). The data from Akiew (1986) were very limited and less robust than those of Nesmith and Jenkins (1985) and so they were not included in the calculations. The experimental conditions used to estimate water potential in ripening peel over the 10 days used a relative humidity of 90–95% (Stratton and von Loesecke 1931). The ripening conditions are less conducive to water loss than the ripening conditions that would be standard practise in Australia and significantly less than distribution conditions when banana fruit are exposed to ambient conditions (see Table 5.7). Based on this information, the model predicts the water potential in ripening banana fruit has a negative effect on *R. solanacearum* where the half life of bacteria in peel is 5.0 days and 1.6 days in the pulp. Based on these data the numbers of bacteria in banana fruit will decrease post ripening and they are unlikely to survive in the pulp tissue. Bacteria in the peel are likely to survive, but in numbers considerably reduced from those estimated for asymptomatic fruit at harvest and packing. For example, with a half life of 5.0 days if 500,000 bacteria per gram of plant tissue are present in the peel of the fruit at the start of ripening then this number will have fallen to 125,000 bacteria when the peel becomes waste after 10 days (Table 5.7). The analysis of (David Turner, Associate Professor, Plant physiologist consultant, pers comm., 25 June 2008) assumes that the bacteria are unable to use the metabolites in the solution surrounding the cells for growth.

In addition to the changes in water potential of banana fruit, the concentration of organic acids in the pulp increases during ripening (Wyman and Palmer 1964) and the pH falls to below 5.0 (Ward and Nussinovitch 1996) at which *R. solanacearum* is unable to grow (Kelman 1953). Another incidental effect of the low pH is that many organic acids are in an undissociated form and are more potent as antibacterial agents than they are at higher pH in dissociated form (Jay et al 2005). Some of the same organic acids found in pulp are also found in peel and among these there are several which have significant antimicrobial activity against a range of Gram-positive and Gram-negative bacteria (Mokbel and Hashinaga 2005). However, given the location of these factors within the symplast (and then mostly in the vacuole), their effect on Moko bacteria (located in the xylem) is likely to be minimal (if at all) while cell membrane integrity and function remains. In addition, as ripening progresses the pH rises as organic acids are used in metabolism, which is a feature of plant tissues undergoing senescence (David Turner, Associate Professor, Plant physiologist consultant, pers comm., 25 June 2008).

In conclusion, growth of the Moko pathogen in fresh green bananas after harvest is unlikely because of the limitations of sub-optimal temperatures and decreasing water potential during the 16–33 day period after harvest and before disposal as waste. The Moko pathogen could survive this period in the cushion and peel but in an attenuated state because of decreasing water potential. Bacteria in the pulp are much less likely to survive due to even lower water potential. Bacteria that have been subjected to a long period under conditions unfavourable for growth usually have a long lag phase before growth resumes when transferred to conditions favourable for growth (Madigan and Martinko 2006).

Survival in banana waste

As the previous section has indicated, a residual population of Moko bacteria in an attenuated state could survive transportation from the Philippines to Australia in the peel tissue while the fruit is intact. This section considers whether the Moko bacteria will survive beyond waste disposal. Banana waste will be discarded within a diverse range of local conditions from urban to rural environments, in composts, controlled waste disposal from retailers or randomly discarded by consumers under a variety of climatic conditions.

Much of the banana waste disposed of to the environment in dry conditions will be subjected to further rapid water loss (Saengpook et al 2007) that will most likely cause sharply decreased water potentials. This would adversely affect the survival of *R. solanacearum* that has a low resistance to desiccation (Kelman 1953). As the waste dries, the Moko bacteria associated with water in the xylem of the peel will become progressively trapped within banana waste.

Under wet conditions, that could prolong the survival of Moko bacteria, re-hydration of the peel will occur. Once this has occurred, bacteria would be washed from the intercellular spaces and remnants of the xylem in the waste (Dong et al 1994). Over time it is doubtful whether the physiological state of the pathogen would enable it to compete for a long period of time with a diverse range of fast growing saprophytic micro-organisms that are adapted to the colonisation of waste material. Kelman (1953) has documented many examples of where *R. solanacearum* under ideal culture conditions fail to compete and survive against a range of soil and saprophytic micro-organisms. In inoculation experiments, *R. solanacearum* was out competed by the secondary micro-organisms if they were present in the cultures (Kelman 1953). However, variability within waste may provide micro-sites where bacteria could avoid the inhibitory effects of saprophytic micro-organisms.

The part played by flood and irrigation water in the survival and dispersal of banana waste of Moko bacteria should take into account the biotic and abiotic factors to which waste would be subject. Bacteria released from banana waste into suspension would be more vulnerable to adverse factors (see *Survival in water*) than bacteria contained within cushion or peel. Previously dry banana cushions would be re-hydrated during flooding and whereever later deposited would be subject to decomposition by fast growing saprophytic micro-organisms, an environment in which Moko bacteria would be uncompetitive for the following reasons.

No information has been found in the literature on the decomposition of discarded banana waste, or the fate of specific pests of concern during the breakdown process. However, many parallel studies have been carried out on the compositional changes occurring in forest litter and crop residues and some guiding principles have been firmly established. Decomposition is a complex process involving a series of successional and overlapping changes in microbial community structure. Many different micro-organisms are involved belonging to both the microflora and microfauna (Warcup 1965) including many detritivores that aid the decomposition of waste by creating entry sites for specialist saprophytic fungi (Paoletti et al 2007).

Banana peel and cushion discarded into the environment serves as a substrate open to colonisation by the microbial community into which it has been introduced. Whether a pest species present in the banana waste is able to compete with the microbial community depends on the growth rate of the pest, its ability to compete for nutrients and its relative response to desiccation, temperature changes and exposure to solar radiation.

The generation time (doubling time) of the Moko pathogen in pure culture under the most favourable laboratory conditions has been estimated as 45–60 min.; for comparison the enteric bacterium *Escherichia coli* has a generation time of 20 min. Many of the mesophilic endospore-forming bacilli present in soils have a similar generation time; some of the thermophilic species present in soils and composts have a generation time shorter than 20 min. (Madigan and Martinko 2006). Because of fluctuations in the physicochemical environment, of temperature, nutrient supply and water activity in particular, growth rates of micro-organisms in nature are well below the maximum growth rates recorded in the laboratory most of the time. Estimates have shown that typical soil bacteria grow in nature at less than 1% of the maximal growth rate observed in the laboratory (Madigan and Martinko 2006). These slower growth rates are also a reflection of the competition between different members of the microbial community. Except for rare instances micro-organisms grow in mixed populations rather than pure cultures in natural environments. A bacterium growing rapidly in pure culture may be a poor competitor with other organisms better suited to the resources and growth conditions available in a particular environment.

According to Lynch and Hobbie (1988 p.110), there is a consistent pattern of microbial succession on crop residues and leaf litter. The primary colonisers are those that can use the simple soluble materials, such as mono and disaccharides that are first to be leached. Fungi such as *Mucor* and *Rhizopus* are

among the primary saprophytes consuming these soluble materials in competition with a great variety of bacteria. The secondary colonizers use more complex materials such as polysaccharides. Many produce cellulases. The tertiary colonisers are capable of metabolizing more complex and resistant polymers such as lignin. The sequence in the primary and secondary colonization of banana waste is unlikely to be radically different from the examples given. The metabolic capability of the Moko pathogen can be matched with the environment of decomposing banana waste. In the primary stage *R.solanacearum* race 2 is well equipped to metabolise hexose sugars, hexose alcohols, and many organic and amino acids (Palleroni and Doudoroff, 1971). Pectate gels are broken down at high, low and intermediate pH (Hildebrand, 1971) and *in planta* other cell wall degrading enzymes, including exo and endoglucanases, play a secondary role in pathogenesis (Denny, 2006). The plant storage polysaccharides starch and inulin are not used as a carbon source by *R. solanacearum* race 2 (Palleroni and Doudoroff, 1971), and no evidence has been found of the ability of the Moko pathogen to breakdown more resistant polymers such as lignin. Micro-organisms with a broad spectrum of degradative activity on complex polymers are widely distributed in soils and decomposing plant material. It is doubtful whether the Moko pathogen would be able to compete either in growth rate or metabolic versatility with these micro-organisms in decomposing banana waste. As the nutritional requirements of Moko in banana waste are modified by decomposition the Moko pathogen will cease to grow while micro-organisms with a broader substrate utilisation will continue to grow.

The fate of the Moko pathogen in banana cushion and peel will also be modified by the site into which the waste is disposed, in compost or on roads, grasslands, recreational land, in shaded or unshaded areas. Compost is likely to be an adverse environment because of competition from a mixed and antagonistic microflora and because the temperatures generated in properly composting material will be lethal over time. Ryckeboer et al. (2002) showed that 12 hours exposure at 52 °C was sufficient to reduce high populations of *R. solanacearum* in an anaerobic composting system to below a detectable level, and 6 hours exposure was sufficient for lower populations.

The surface temperature to which banana waste is exposed will depend upon many factors: soil type, plant cover, the amount of rainfall and degree of exposure to solar radiation. There are gross differences in diurnal temperature range between moist shaded areas and those subjected to full sun exposure. Results representative of temperature variations that can occur were obtained in an almond orchard in California's western Fresno County (Danyluk et al 2007). Surface soil temperatures under the tree canopy and in the middle of the drive row were measured at midday from June to September 2004 and were also monitored at about every two hours on a representative summer day. The midday surface soil temperature under the tree canopy ranged from 20 – 35 °C and at the centre of the drive row between 35 and 55 °C. Over a 24 h period the sheltered soil surface temperature ranged from 15 – 24 °C and the exposed soil temperature from 16 – 54 °C. The temperature from about 12 noon rose from 45 °C to 54 °C and decreased to about 45 °C by 2.00 p.m. Similar temperature variations would be expected to occur under Australian conditions in the summer; in some localities a lower range would be expected and in others a higher range. Temperatures within the range of 45 – 55 °C for 10 minutes are lethal for *Ralstonia solanacearum* (Smith 1914; Kelman 1953). Banana waste exposed to such temperatures will rapidly desiccate. It is unlikely that the Moko pathogen in waste would survive the combined effect of desiccation and high temperature for periods greater than 12–48 h. However, under shaded conditions desiccation will occur but at a slower rate; survival would be expected to be more prolonged. Under conditions of heavy precipitation, decomposition of banana waste would ensue with the successional changes in microflora previously described.

In contrast to the likely competitive ability of the Moko pathogen in banana waste, Greene and Goos (1963) have reported the development of rot and mould causing organisms in freshly cut crown surfaces of banana hands, a condition referred to as crown rot, as a serious problem encountered in shipments of boxed bananas imported from Central and South America into USA. A wide variety of fungi have been frequently isolated including, *Fusarium roseum*, *Verticillium theobromae* and *Gloeosporium musarum*. Other fungi found included *Mucor* spp., *Myrothecium* spp., *Pestalotia* spp., *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., *Deightoniella torulosa Fusarium moniliformae*, *Nectria* spp., *Monilia* spp., *Stachylidium bicolour*, *Curvularia pallescens* and *Trichoderma viride*. All the fungi associated with crown rot of bananas as reported by Greene and Goos (1963) are recorded

present in Australia (Rippon 1972; APPD 2008). It is possible that these fungi contaminated the cut surface from inoculum present on the fruit in the field or during washing fruit prior to packing. Similarly, a high incidence of crown rot, caused by several fungal genera, has been detected on bananas grown without the use of chemicals in the Philippines (de Lapeyre de Bellaire and Mourichon 1997; Alvindia et al 2002**).** These authors have demonstrated, from artificially inoculated experiments, that several fungi or a mixture of fungi were capable of causing rots in banana crowns/cushions. In general, activity of all the pathogens causing rots was retarded at lower temperatures (14.4 °C) and enhanced at higher temperatures (23.9 °C), with moderate to severe rotting of tissue within 6 days after inoculation. Therefore, it can be assumed that many of the fungi shown above and other micro-organisms present in the soil would assist in the decay of banana cushions discarded as waste within a short period.

Jay et al (2005) have given the percentage composition of the water, carbohydrate, protein, fat and ash content of some common fruits including bananas. The average water content in 18 common fruits is about 85%, whereas in banana pulp the total water content is 75%, which is the lowest for any of the fruits. These authors have indicated "On the basis of nutrient content, these products appear to be capable of supporting the growth of bacteria, yeasts and moulds. However, the pH of fruits is below the level that generally favours bacterial growth. This one fact alone would seem to be sufficient to explain the general absence of bacteria in the incipient spoilage of fruits. With the exception of pears, which sometimes undergo *Erwinia* rot, bacteria are of no known importance in the initiation of fruit spoilage."

The moisture, dry matter (DM) and nutrient content of banana in the environment are important during the process of decay. Essien et al (2005) have shown that banana fruit peel of *Musa sapientum* has a moisture content of 78.4%, carbohydrate content of 59.51% DM, protein content of 7.87% DM, crude fat content of 11.60% DM, crude fibre content of 7.68% and a dry matter content of 14.08%. The total ash content of the peel was 13.44% DM. The water content for hard green bananas cv Williams has been recorded to be 92% (David Turner, Associate Professor, Plant physiologist consultant, pers comm., 25 June 2008). Essien et al (2005) have indicated that the high carbohydrate content could serve as the main carbon source for microbial growth, high protein content will support mould growth, fats are used as alternative energy source, the low fibre content may favour fastidious organisms, ash which reflects the amount of mineral elements (rich in potassium, phosphorus, and magnesium and sodium) and some of which serve as prosthetic groups of some enzymes. The presence of ascorbic acid indicates inhibitory properties of the substrate against bacteria.

Ambuko et al (2006) have shown that unripe banana fruit contains as much as 70–80% starch on a dry weight basis, which is reduced to less than 1% at the end of the climacteric period, while sugars, mainly sucrose accumulate to more than 10% of the fresh weight of the fruit.

It has also been demonstrated that water-soluble pectin, pectinmethylesterase (PME) and pectin lyase (PL) was higher at the rupture area of the peel near the crown when humidity was higher (Saengpook et al 2007), suggesting that tissue degradation is quicker under humid conditions.

In summary, the Moko pathogen in banana waste would not be competitive because of its attenuated state after importation, relatively slow growth rate, lack of nutritional versatility and inability to cope with the stresses of exposure to solar radiation, desiccation and moderately high temperatures where it is likely to be restricted to the vascular tissue of the waste in dry conditions. In compost, the heat generated by micro–organism metabolism will kill low numbers of Moko bacteria in hours. Under wet conditions that favour saprophytes, the competition from a diverse microbial community growing in banana waste is likely to include members which produce lytic enzymes and antibiotic substances harmful to the Moko pathogen. Taking these factors into consideration, the survival of the Moko pathogen in banana waste will be limited to a very short period of time.

Incidence of Moko disease in the Philippines

It has been reported that all Cavendish banana plantations in the Philippines are subject to weekly surveys for Moko disease and also that Cavendish banana plantations are harvested on a weekly cycle

(BPI 2002b). Harvesting occurs independently of Moko disease surveys, so the incidence of the disease in plants at harvest would be similar to that in the plantation as a whole.

When a diseased plant is found, this plant is destroyed along with the six surrounding plants. This is recorded as a case of Moko disease. It is not known how many of the visibly healthy plants in each Moko case are infected, although it is apparent that the need for such a control measure is based on an expectation that one or more of these plants could be infected. Correll (2004) suggests that there is probably at least one additional infected plant amongst the six additional plants that are removed with each case of Moko disease.

Evidence of the incidence of Moko disease was observed by the chairs of the Technical Working Group during their visit to the Philippines in August 2001 (BA 2002c). Aerial photographs taken at the time illustrated a scattered, apparently random distribution of spots within a Cavendish banana plantation where cases of Moko disease and surrounding plants had been removed. Additional spatial data on disease incidence has not been provided, particularly in regard to any gradient effect of Moko disease incidences in Cavendish bananas adjacent to small holdings affected by Bugtok disease.

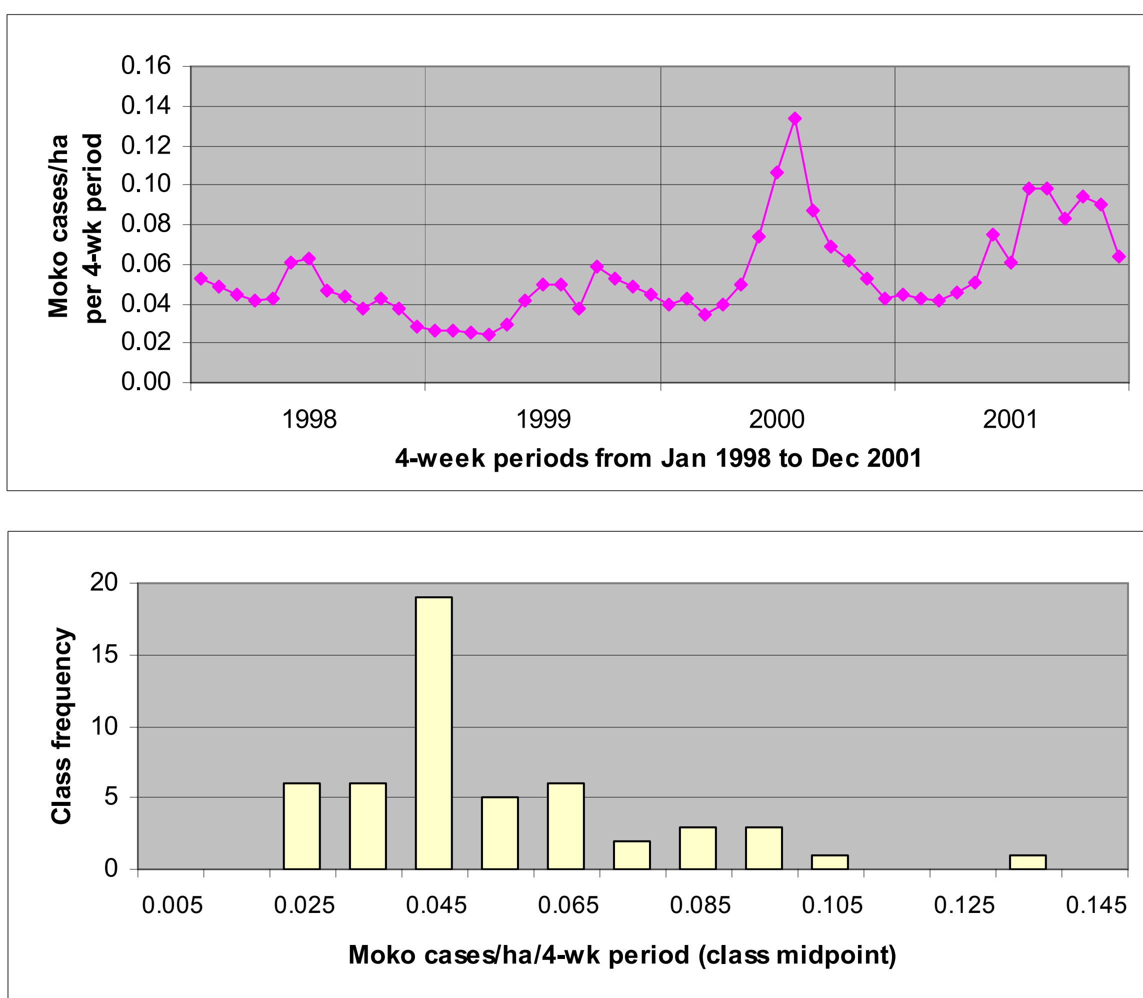


Figure 5.5 Incidence of Moko disease in Cavendish banana plantations in the Philippines

Data provided by Philippines authorities (BPI 2002b), presented as cases per hectare per four-week period (top) and as a frequency distribution of the incidence data (bottom).

Survey data supplied by Philippine authorities (BPI 2002b) provide a summary of the average number of Moko cases per hectare for each four-weekly period in the years 1998–2001 (Figure 5.5). Details have not been provided of the plantations from which these data were derived, but it is evident from the provided data that the disease incidence varies over time, with successive periods of increasing and decreasing incidence. Peaks of disease incidence were apparent in the middle to second half of each year. Analysis of the incidence data shows a mode of approximately 0.05 cases per hectare per four-

week period, with a range from a minimum of 0.024 and a maximum of 0.134. The mean incidence was 0.054 cases per hectare per four-week period.

Control measures

Banana plants that are systemically infected with *R. solanacearum* race 2 will eventually succumb to the disease, as the pathogen cannot be eliminated without killing the host (Thwaites et al 2000). Control of Moko disease is considered from two aspects; measures which are applicable in the field, and sanitation measures which are applied to banana bunches immediately after dehanding in the packing house.

Pre-harvest control

Current pre-harvest control measures are based on sanitation and spot eradication, including:

- early detection and monitoring for presence of the pathogen
- preventative management measures that exclude/minimise:
    - insect visitation by de-belling (that is, removal of the male flower bud from the peduncle), and
    - infection and spread through contaminated soil
- swift control by destruction of infected plants or mats generally by glyphosate injection (Black and Delbeke 1991)
- the use of clean planting material.

In circumstances where the pathogen has entered the soil, the general control method in the Philippines is based on heat treatment, whereby infected plant material is burnt together with rice husks within the infected area (BPI 2001). Bare fallow is recommended after heat treatment, including maintenance of a buffer zone around the treated area. Replanting after soil fumigation may commence three weeks after treatment. Alternatively, the area is fallowed for 18–24 months prior to replanting. During fallow, the area is kept free from weeds (BPI 2001).

Regular field surveillance, spot eradication and plant maintenance operations (that is, deflowering and bagging of the developing fruit bunches) have kept incidences of *R. solanacearum* race 2 infection in commercial Cavendish banana plantations at a low incidence rate world wide. Replanting of Central American plantations commences at a 5% disease incidence – that is, at a point where the sanitation of plantations is no longer economically viable (Lehmann-Danzinger 1987).

Despite the general lack of detailed quantitative investigation of *R. solanacearum* race 2 in soil, there is agreement that the pathogen can persist for 12–18 months in soil (Sequeira 1962; Stover 1972). Sequeira (1962) reported that Moko disease was effectively controlled when infested soils were fallowed for 24 months. Weed fallowing was as effective as rotation with tropical kudzu. Shallow tilling during dry weather (Sequeira 1958) and good drainage (Lozano et al 1968) of soils further reduce the necessary fallow period for effective disease control.

Shallow tilling eliminates live plant roots, interrupts the water capillary system and exposes contaminated soil layers to sunlight and higher temperatures at the soil surface, to which *R. solanacearum* race 2 is highly susceptible (Kelman 1953; Sequeira 1962, 1993). Barren soils and flooding have been demonstrated to be effective in the eradication of soilborne inoculum (Hartman and Elphinstone 1994), but are rarely feasible. Mixed fertiliser applications, which included compost, effectively reduced the pathogen in the soil in research conducted in India (Roy et al 1999). Bioremediation products (that is, suspensions of live, antagonistic micro-organisms) that specifically aim at the elimination of *R. solanacearum* race 2 from the soil are currently not available in Australia.

Post-harvest control

Post–harvest, many factors contribute to losses in fresh fruits and vegetables. These include disease causing organisms and mechanical damage during harvesting and handling, which could also

predispose the product to secondary infection. Post–harvest control measures are targeted at a range of surface contaminants and do not affect the survival of pests that are within the banana fruit.

In the Philippines banana industry the post-harvest treatment of banana bunches after de-handing involves flotation in a water tank containing 20 ppm chlorine and 200 ppm (= 0.02% or 200 mg per L) of alum solution for a period of 25 minutes. The chlorine acts as a sterilizing agent and the primary function of alum treatment is to coagulate or flocculate the latex which exudes from the cushion end of harvested fruit. According to information provided by the Philippines industry (Bureau of Plant Industry 2002a) there is a second treatment with chlorine plus alum after the flotation process. Hands/clusters of fruit to a weight of 12.5 kg are collected on a tray and then treated by spraying with a solution containing chlorine (10%) and alum (1%) prior to packing.

Chlorine is a very potent disinfectant with powerful oxidising properties. It is soluble in water, either by injection of chlorine gas (used in large operations) or by addition of hypochlorite salts (calcium or sodium). This solution of chlorinated water consists of a mixture of chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions (OCl^-) in amounts that vary with the pH (Suslow 1997). The amount of chlorine available for oxidative reaction and disinfection is referred to as available chlorine. Hypochlorous acid is the form of free available chlorine that has the highest biocidal activity against a broad range of micro–organisms (Parish et al 2003). It is both a strong oxidant and an effective biocide while hypochlorite ion (OCl^-) is a weaker oxidant and a very poor biocide (Kelly 2004).

Chlorine-based chemicals are perhaps the most widely used sanitiser in food processing for several decades (Walker and LaGrange 1991; Cherry 1999). Chlorine is known to have strong biocidal properties against a wide range of organisms. It is highly effective against non-spore forming bacteria, but also to a lesser extent against spore-forming bacteria, fungi, algae, protozoa and viruses (Dychdala 1991). Chlorine is also used as a disinfectant in drinking water and washing applications, to reduce the surface microbial contamination of food products and to sanitise processing facilities in packing operations (Dychdala 1991; Beuchat 1992; Parish et al 2003). These authors have indicated that chlorine treatments are usually targeted against organisms that cause spoilage and affect human health during post–harvest handling of fruit and vegetables.

The efficacy of chlorine is influenced by a number of factors. These include the chlorine concentration and exposure time, pH, temperature and organic matter content. The type of product disinfected and the local conditions will determine the concentration and exposure time of chlorine. Some bacterial populations are killed at 5 ppm of available chlorine (Somers 1951), but most require a 50 to 200 ppm with a contact time of 1 to 2 minutes (Parish et al 2003). These authors indicate that a similar concentration of chlorine is generally required to sanitise processing equipment. In aqueous solutions, the equilibrium between HOCl and OCl^- is pH dependent with concentrations of HOCl increasing as pH decreases. Typically, pH values between 6.0 and 7.5 are used in sanitiser solutions to maximise chlorine efficacy and minimise corrosion of equipment (Parish et al 2003). Above pH 7.5, very little chlorine exists as active HOCl, but rather as far less active OCl^-. Below pH 6.0, noxious Cl_2 is formed. Suslow (1997) indicates that the activity of chlorine increases with temperature resulting in loss of chlorine to the atmosphere due to reactions with organic matter. Similarly disinfection efficacy was reduced by low temperature and improper pH values in hydrocooling. Chlorine is highly reactive with organic matter such as fruit, leaves or soil in the presence of oxygen. Organic matter in water will inactivate HOCl and quickly reduce the amount of available chlorine, and therefore the overall antimicrobial activity.

Chlorine gas (Cl_2) formed at low pH escapes from solution resulting in the release of strong noxious chlorine odours into the packing house. In addition, a majority of the chlorine added to a dirty dump tank is transformed into chloramine through reactions with natural amine-containing organic molecules in the water. Chloramines are much more volatile than chlorine, and are also readily released at reduced pH (Kelley 2004). Chlorine gas is extremely dangerous and should be used only in properly designed containment facilities that are isolated from the packing house. Chlorine fumes released from treated water will cause worker discomfort and eye irritation. According to Parish et al (2003) 'safety concerns about the production of chlorinated organic compounds, such as trihalomethanes, and their impact on human and environmental safety have been investigated. At the

food service and household levels, chlorine remains a convenient and inexpensive sanitiser for use against many food-borne pathogens.'

To ensure effective control of micro–organisms, the chlorine concentration and the pH should be monitored at regular intervals, especially when large quantities of produce are being processed. Excessive chlorine reaction odour or chlorine gas may also indicate improper pH adjustment. Changing chlorinated water frequently or filtering out organic matter and debris is essential for effective sanitation (Suslow 1997).

Alum solutions have two effects that are likely to affect the fate of bacteria suspended in wash water. Alum at a concentration of 10-30 mg per L is used to remove suspended particles during purification of waste water. Coagulation and flocculation are the mechanisms by which particulate and colloidal materials are removed from water in the process of clarification. Coagulation involves charge neutralization that results in the destabilization of suspensions of particles in the colloidal size range of 1-500 nanometres allowing agglomeration to occur. Colloidal particles have a negative electrical charge; this net negative charge results in stable suspensions due to the repulsive forces between each particle.

The extent of particle agglomeration due to coagulation cannot be predicted. In some circumstances, very small numbers of particles may agglomerate to form microfloc. The coagulated material may or may not be agglomerated enough to achieve good settling without flocculation. A high density macrofloc may not need further treatment, while microfloc and low density macrofloc will likely require flocculation for optimum clarification.

Flocculation can be defined as the mechanism by which microfloc or low density macrofloc particles are further agglomerated resulting in rapid settling floc bodies and enhanced finished water quality.

Coagulation-flocculation followed by sedimentation, filtration and disinfection are processes used world wide in the water treatment industry. Inorganic substances are used as coagulants and of these alum is by far the most commonly used. Trivalent cations such as aluminium and ferric iron coagulate colloidal suspensions by charge neutralization and by promoting agglomeration. In addition to their coagulating ability, they are also capable of further flocculation through their ability to form hydrated gelatinous hydroxides at appropriate pH levels. These gelatinous hydroxides entrap destabilized particles as they sweep through the water under the force of gravity.

Gram-negative bacteria such as the Moko pathogen are cells (or particles) at or just above the upper limit of the colloidal size range (500 nanometres). All Gram-negative bacteria have a net negative surface charge. The negative charge derives in part from the phosphate groups which are present in the lipopolysaccharide component of the outer membrane that is a defining feature of the cell wall of all Gram-negative bacteria. Gram-negative bacteria are commonly enveloped by discrete capsules or diffuse slime layers consisting of homo- and/or heteropolysaccharides. The latter are commonly acidic in nature because of the dissociated carboxyl groups present in most heteropolysaccharides.

A second biological effect of aluminium salts derives from the toxicity of the trivalent aluminium cation (Al^{3+}). Aluminium is a metal lacking biological function and accordingly belongs to the class of non-essential chemical elements. Evidence from various sources has shown that aluminium salts are toxic to micro–organisms. Toxic effects are the result of competition with iron or magnesium, binding to DNA or binding to cell walls or membranes with disruption of transport functions (Pina and Cervantes, 1996).

Interpretation of the results of experiments on the effects of aluminium on micro–organisms have been difficult to interpret, because of the complex chemistry of aluminium, which polymerises, interacts with phosphates and organic acids, and acidifies culture media (Pina and Cervantes, 1996).

There is general agreement that one of the most important mechanisms of aluminium toxicity is its replacement of divalent metal complexes, chiefly magnesium (Mg^{2+}) and calcium (Ca^{2+}), in cells or membranes, but there is uncertainty as to whether the effect is generic to all forms of soluble aluminium. Most studies suggest that monomeric Al^{3+} is the most actively toxic inorganic form of

aluminium, even though toxicity seems to peak in the slightly acidic to neutral region (pH 5 to 6.5) rather than at lower pH's, where Al 3+ dominates (Amonette et al. 2003).

Illmer and Erlebach (2003) identified a distinct inhibition of the growth of a typical Gram-positive soil bacterium by aluminium. The content of intracellular water and consequently the cell size of the bacterium significantly increased when cells came into contact with aluminium. Both parameters were also increased when applying hydrogen chloride (HCl), but aluminium caused the greater change, so that discrimination between the effects of aluminium on the one hand and a sole pH effect on the other hand could be made. The authors concluded that the probable mechanism of aluminium-toxicity in their bacterium could be attributed to an osmo-regulatory disorder connected with a malfunction of the cell membrane and cell wall. Later work with a Gram-negative bacterium (*Erwinia carotovora* subsp.*atroseptica*) showed that exposure to aluminium chloride (0.05, 0.1 and 0.2 M concentrations) for 0 – 20 min brought about ultrastructural changes. Bacteria showed loosening of the cell walls, cell wall rupture, cytoplasmic aggregation, and an absence of extracellular vesicles. Bacterial mortality was evident when bacteria were exposed to either a high concentration (0.2 M) of aluminium chloride or prolonged exposure (20 min) to 0.05 M aluminium chloride or to a pH of 2.5. Bacteria exposed to lower concentrations of aluminium chloride (0.05 M and 0.1 M) for 10 min or less did not show evidence of membrane damage. The authors concluded that mortality caused by aluminium chloride involves membrane damage and subsequent cytoplasmic aggregation (Yaganza et al. 2004).

Alum solutions have been widely used to extend the vase life of cut flowers that are prone to senesce a few days after placement in vase water. Many cut flowers show wilting and bending of the floral axis just below the flower head within a few days of placement in vase water. Water stress and wilting is the result of vascular occlusion and alterations in the transpiration rate through stomata of flowers, stems and leaves. Several factors contribute to the short vase-life of cut flowers. Bacterial growth in the vase water and in and on cut stems is one major factor. According to van Doorn (1997):

> "When cut flowers are placed in water directly after harvest, their xylem becomes blocked by bacteria growing at the cut surface and inside the xylem conduits. This bacterial blockage apparently is a physical phenomenon, due to the presence of living bacteria and the extracellular polysaccharides they produce and to dead bacteria and their degradation products. The bacterial blockage may be followed by cavitation in the xylem, that is, the filling of the conduits with gas. The bacterial blockage probably develops in the basal stem end of all cut flowers, but some are much more sensitive to this blockage than others."

In many cut flowers the suppression of microbial growth in the vase solution results in a delay of wilting. Various organic and inorganic supplements to vase water have been evaluated for their antimicrobial activity. Some are effective only at concentrations which are toxic to cut flowers. Antimicrobial compounds that delay wilting without being toxic to cut flowers include salts of copper, zinc, cobalt, nickel and aluminium (van Doorn, 1997). Anhydrous aluminium sulphate at concentrations of 50 to 100 ppm has been used in many preservative solutions for roses and other flowers (Halevy and Mayak, 1981; Mohan Ram and Rao, 1977). Part of the effect of aluminium sulphate solution is attributable to acidification of the water which improves water uptake and depresses microbial growth including direct germicidal properties . Van Doorn et al. (1990) tested the efficacy of various antimicrobial compounds, including aluminium sulphate, for their effect on the number of bacteria in stems of cut rose flowers. All compounds tested decreased the number of bacteria in stems of cut roses with respect to untreated controls.

According to Ichimura et al. (2005):

> "To inhibit bacterial proliferation, germicides, such as silver nitrate, aluminium sulphate and 8-hydroxyquinoline sulphate (HQS) have been used in commercial preservatives. These germicides inhibit vascular occlusion and extend the vase life of cut rose flowers."

Liao et al (2001) found that a floral preservative solution containing aluminium sulphate at a concentration of 150 mg per L extended the vase life of cut flowers of *Eustoma grandiflorum.* The vase life was about 15 days for cut flowers supplied with aluminium sulphate, but only 8 days in the water controls.

Recent work has illustrated the potential of alum solutions as a post-harvest dip to prevent the onset of bacterial soft rot caused by *Erwinia carotovora*. McGuire and Kelman (1984) examined the effect of infiltration of potato tubers with 0.08 M solutions of various monovalent, divalent and trivalent salts. The monovalent cations sodium and potassium were least effective in reducing the amount of bacterial soft rot. Strontium (Sr^{2+}), Mg^{2+} and Ca^{2+} produced a significantly greater response, and of these cations Ca^{2+} was the most effective. The greatest reduction in soft rot was obtained with aluminium nitrate solution. However, the authors concluded that Al^{3+} ions at the concentration used are toxic to bacteria and the host response may not have been as great as it appeared to be.

Mills et al (2006) obtained convincing evidence of the efficacy of several salt compounds at concentrations of 0.002 M, 0.02 M and 0.2 M. Alum was one of several salts which inhibited soft rot bacteria in agar media at all concentrations used. Alum was also effective as a preventative or curative post-harvest treatment to reduce the effect of bacterial soft rot in stored potato tubers.

In summary, chlorine solutions have been used extensively for the post-harvest treatment of fruit and vegetables. Chlorine treatment suffers from the disadvantage that activity is diminished in the presence of organic matter and chlorine solutions are corrosive and chlorine gas evolved is highly toxic. Alum solutions promote flocculation and coagulation and remove particulate material, including bacteria, from suspension during waste water treatment. There is a long history of the use of metal salts such as alum in vase water to increase longevity of cut flowers, and the effect is attributable at least in part to the antimicrobial effect of trivalent aluminium. Alum solutions have been shown to be effective as a post-harvest dip to reduce the effect of bacterial soft rot in potatoes in store and show promise as a sanitising agent.

Appendix 6. Black Sigatoka

Scientific name

Teleomorph

Mycosphaerella fijiensis M Morelet 1969 [Dothideales: Mycosphaerellaceae]

Synonym

Mycosphaerella fijiensis var. *difformis* JL Mulder and RH Stover 1976
[Dothideales: Mycosphaerellaceae]

Anamorph

Pseudocercospora fijiensis (M Morelet) Deighton 1976

Synonyms

Cercospora fijiensis M Morelet 1969
Cercospora fijiensis var. *difformis* JL Mulder and RH Stover 1976
Paracercospora fijiensis (M Morelet) Deighton 1979

Andromorph

Asteromella sp.

Common names

Black Sigatoka (Stover 1974) or black leaf streak (Rhodes 1964). Both names have been accepted by the International Society for Plant Pathology – Committee on Common Names of Plant Diseases (Jones et al 2006).

Hosts

Hosts of *M. fijiensis* are primarily species of *Musa* (Eumusa) and in particular, hybrids of *M. acuminata* (A) and *M. balbisiana* (B) in the AA, AAA, AAAA, AAB and ABB genomic subgroups (Meredith and Lawrence 1970; Gauhl 1994; Carlier et al 2000). Cultivars within these groups show varying degrees of resistance, evident in some cases as a slow lesion development and in other cases as a rapid hypersensitive response to infection (Meredith and Lawrence 1970; Fouré 1994; Beveraggi et al 1995). Ortiz and Vuylsteke (1994) found evidence that black Sigatoka host resistance was determined by one major recessive allele and two independent alleles with additive effects.

Symptoms of black Sigatoka caused by *M. fijiensis* have been recorded on the wild banana species *Musa acuminata* subsp. *banksii*, *M. acuminata* subsp. *burmannica*, *M. acuminata* subsp. *malaccensis*, *M. acuminata* subsp. *microcarpa*, *M. acuminata* subsp. *zebrina*, and on *M. balbisiana* (Gauhl 1994; Carlier et al 2000). Most wild species of *Musa* that have been tested have been found to become infected, but invasion has been halted at a very early stage by a hypersensitive response (Carlier et al 2000). It is not known whether the Australian species *M. fitzalanii* (listed under the *Endangered Species Protection Act 1992*) and *M. jackeyi* are susceptible. Gauhl (1994) reported field surveys of a wide range of potential hosts of *M. fijiensis* in the Zingiberales order. No disease symptoms or development of fungal fruiting bodies were found on *M. textilis*, *M. coccinea*, *M. velutina*, *Ensete* sp., *Heliconia* sp., or any species other than those listed as being susceptible above. However, Gasparotto et al (2005) reported that a leaf disease of *Heliconia psittacorum* in Brazil was caused by fungus apparently identical to *M. fijiensis*. The fungus isolated from the leaf tissue proved to be pathogenic to

both *H. psittacorum* and banana cv Prata Anã (AAA). It produced conidiophores and conidia on diseased heliconia leaves, although pseudothecia were not observed (Gasparotto, Plant Pathologist, Empresa Brasileira de Pesquisa Agropecuaria, pers comm 30 November 2007). No records have been found of *M. fijiensis* on any *Heliconia* sp. in the Philippines or other parts of the world.

The reactions of Australian banana and plantain cultivars to *M. fijiensis* have been studied in off-shore arboreta (Carlier et al 2000) but little is known about the susceptibility of other plants of the order Zingiberales grown in Australia, nor have data been provided on the susceptibility of these plants in the Philippines.

Plant parts affected

Mycosphaerella fijiensis infects and causes disease primarily on the leaf lamina (Meredith 1970; Carlier et al 2000). It infects the surface tissues of fruit to a limited extent, causing initial speck symptoms (Table 6.1) on plantain cv. Cavendish Harton (Cedeno et al 2000) and banana cv (Fullerton 2006). In each case, mycelia were observed in the infected fruit tissue and *M. fijiensis* was identified by conidial morphology in culture and by PCR analysis. The symptoms on fruit did not progress to the first streak stage (Table 6.1) and the production of conidiophores on the fruit surface was not described. Cedeno et al (2000) reported that conidiophores, conidia and ascospores were collected with adhesive tape from fruit surfaces in the field. However, the evidence for sporulation of *M. fijiensis* on infected fruit tissue is not conclusive because the possibility of surface contamination from alternative sources was not excluded.

Mycosphaerella fijiensis has not been reported to infect the flowers, flower bracts (bell), mature leaf bases (pseudostem), corm or roots of Cavendish bananas (Meredith 1970; Carlier et al 2000). However, there appear to be no studies specifically directed at finding infections on these tissues and the question of whether or not these tissues are susceptible to infection remains open.

Six different stages of disease development have been identified (Meredith and Lawrence 1969; Fouré et al 1987) and are summarised in Table 6.1. Often, all stages of disease can be seen on a single leaf (Meredith and Lawrence 1969).

Table 6.1 Symptoms of black Sigatoka infection of Cavendish banana leaves

Source: Meredith and Lawrence (1969) and Fouré (1987)

Meredith and Lawrence	Fouré Stage	Description
–	1a	Minute whitish or yellow depigmentation mark on lower surface of leaf. These appear 14–20 days after infection.
Initial speck stage	1b	Red-brown speck on lower surface. Conidiophores are produced from this stage onwards.
First streak stage	2	Red-brown streaks about 20 x 2 mm, on upper and lower surfaces of the leaf.
Second streak stage	3	Streaks wider than in Stage 2, colour changing to dark brown. Spermogonia are formed from this stage on.
First spot stage	4	Dark brown (lower surface) to black (upper surface) spots.
Second spot stage	5	Black spots with chlorotic halo; lesion becoming depressed. Pseudothecia are formed from this stage on. Conidiophore production ceases.
Third or mature spot stage	6	Spot is surrounded by a dark brown to black border. Centre of lesion dries out, turns grey to whitish and becomes further depressed. Pseudothecia begin to mature.

Carlier et al (2000) note that, although all leaves seem to be equally susceptible to infection by *M. fijiensis* regardless of age, most infections occur on new leaves between emergence and unfurling. Jacome and Schuh (1992) inoculated small tissue-cultured plants in growth cabinets and found that infection was more severe on the older leaves. However, Leach (1946) noted in the field that infection of older leaves by *M. musicola* was inhibited by epiphytic microflora that developed abundantly under tropical conditions. This inhibition could be overcome experimentally by wiping the leaf surface clean before inoculation. Pont (1960a) inoculated the under surfaces of Cavendish banana leaves in the field

with a standard inoculum of *M. musicola* conidia and found that fully expanded leaves from leaf 1 (youngest) to leaf 5 could be infected but the density of streak lesions decreased with increasing age of the leaf (Figure 6.1). Very few infections were obtained when the under surfaces of leaves 6-7 were inoculated, or when the upper surface of any leaf was inoculated.

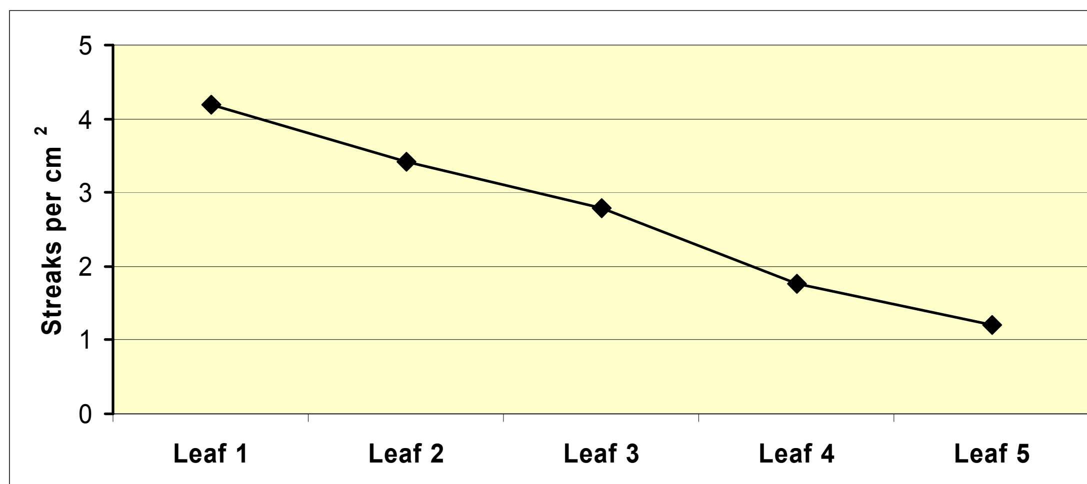


Figure 6.1 Infections on leaves 1-5 inoculated with *M. musicola conidia*

Source: from Pont (1960a)

The time between infection and the appearance of symptoms varies with weather conditions, inoculum density and plant susceptibility. Spots develop more quickly and in greater numbers when large numbers of spores are inoculated onto leaves than when small numbers are used (Leach 1946; Carlier et al 2000). In plantations where the disease is not managed, black Sigatoka streaks appear on leaves 2–4 and spots and streaks appear on leaves 5–6 (Meredith and Lawrence 1969). Whole sections of leaves can become necrotic as spots coalesce. It usually takes three to four weeks after symptoms first appear for the leaf to die, although this time may be shorter when there is severe infection (Meredith and Lawrence 1969; Gauhl 1994).

The effects of infection in susceptible cultivars are to reduce the production of assimilates needed for optimal growth and fruit production (Carlier et al 2000). Severely affected plants may have fewer than six functional leaves on unbunched stems, while all leaves may die on a bunched stem before the bunch is harvested (Meredith and Lawrence 1969; Carlier et al 2000).

The number of disease-free leaves, disease scores of specific leaves (third leaf) and average disease scores for whole plants (leaves 1–8) provide easily assessed reproducible estimates of black Sigatoka disease severity in banana plantations. Various scales for estimating disease severity and incidence are used (for example, Meredith and Lawrence 1970; Stover and Dickson 1970; Stover 1971; Vawdrey et al 2004).

Distribution

Black Sigatoka has been recorded in 72 countries in Central and South America, Oceania, South–East Asia and parts of Africa (Table 6.2). Notable exceptions where bananas are grown commercially are India, Pakistan, South Africa, Israel and mainland Australia.

Table 6.2 Distribution list for black Sigatoka

Country	References
American Samoa	Firman (1975)
Australia	restricted distribution (see text)

Country	References
Belize	Stover (1980)
Benin	Jones and Mourichon (1993)
Bhutan	Carlier et al (2000)
Bolivia	Carlier et al (2000)
Brazil	Carlier et al (2000)
Burundi	Mourichon and Fullerton (1990)
Cameroon	Carlier et al (2000)
Central African Republic	EPPO (2004)
China	Jones and Mourichon (1993)
Colombia	Carlier et al (2000)
Comoros	Jones and Mourichon (1993)
Congo	Carlier et al (2000)
Congo Democratic Republic	Carlier et al (2000)
Cook Islands	Firman (1975)
Costa Rica	Stover (1980)
Cuba	Vidal (1992)
Dominican Republic	EPPO (2004)
Ecuador	Mourichon and Fullerton (1990)
El Salvador	Mourichon and Fullerton (1990)
Fiji	Rhodes (1964)
French Polynesia	Graham (1968)
Gabon	Frossard (1980)
Ghana	Carlier et al (2000)
Guadeloupe	EPPO (2004)
Guatemala	Stover (1980)
Guinea-Bissau	EPPO (2004)
Guyana	EPPO (2004)
Haiti	EPPO (2004)
Honduras	Carlier et al (2000)
Indonesia	Carlier et al (2000)
Ivory Coast	Mourichon and Fullerton (1990)
Jamaica	Carlier et al (2000)
Kenya	Carlier et al (2000)
Madagascar	Jones (2003)
Malawi	Carlier et al (2000)
Malaysia	Graham (1968)
Mauritius	EPPO (2004)
Martinique	EPPO (2004)
Mexico	Stover and Simmonds (1987)
Micronesia (Federated states of)	Graham (1968)
Netherlands Antilles	EPPO (2004)
New Caledonia	Graham (1968)
Nicaragua	Stover (1980)
Niger	EPPO (2004)
Nigeria	EPPO (2004)
Niue	Stover (1976)
Norfolk Island	Jones (2000)
Panama	Stover and Simmonds (1987)

Country	References
Papua New Guinea	Stover (1976)
Peru	Jones (2003)
Philippines	Hapitan and Reyes (1970); Carlier et al (2000)
Rwanda	Mourichon and Fullerton (1990)
Samoa	EPPO (2004)
Sao Tome et Principe	EPPO (2004)
Sarawak	EPPO (2004)
Singapore	Graham (1968)
Solomon Islands	Stover (1976)
Surinam	EPPO (2004)
Taiwan	EPPO (2004)
Tanzania	Carlier et al (2000)
Thailand	Carlier et al (2000)
Togo	Mourichon and Fullerton (1990)
Tonga	Johnston (1965)
Uganda	Tushemereirwe and Waller (1993)
USA	Meredith and Lawrence (1969)
Vanuatu	Graham (1968)
Venezuela	Jones and Mourichon (1993)
Vietnam	Jones and Mourichon (1993)
Wallis and Futuna Islands	EPPO (2004)
Zambia	EPPO (2004)

Philippines

Black Sigatoka was recorded on the Philippine islands of Luzon in 1964 (Hapitan and Reyes 1970) and subsequently throughout most of the Philippines (Hapitan and Reyes 1970; Carlier et al 2000).

Australia

Black Sigatoka was detected on several Torres Strait islands between Australia and Papua New Guinea and on the northern tip of Cape York Peninsula in 1981 (Jones and Alcorn 1982). It is thought to have been present in this area for a considerable time before 1981 (Allen and Peterson 1999). Attempts to eradicate the pathogen were not successful and the disease has since been detected in the Torres Strait area on a number of occasions (Allen and Peterson 1999). Black Sigatoka was not detected elsewhere on the Cape York Peninsula or mainland Australia in 1981.

From 1981–2001 black Sigatoka was detected on seven occasions in areas of Cape York Peninsula extending from Bamaga in the north to Daintree in the south (Allen and Peterson 1999). Each of these outbreaks was treated by destroying all host plants and prohibiting the replanting of susceptible banana varieties until there was confidence that the organism had been eradicated. One of these outbreaks was in a commercial Cavendish banana plantation approximately 35 hectares in area.

Black Sigatoka was found in the banana production area at Tully, north Queensland, in April 2001. Between April and November 2001, black Sigatoka was found in 13 commercial plantations and 12 clumps of unmanaged banana plants (Peterson et al 2003). An emergency response was mounted in accordance with a previously agreed contingency plan (Allen and Priestly 1999). Restrictions were introduced on the marketing of fruit produced within 50 km of any diseased plants and all host plants within 500 m of any diseased plant were destroyed. The quarantine distances of 50 km and 500 m for market access and destruction, respectively, were based on considerations of the dispersal characteristics of the pathogen (see below).

The initial responses to the disease detection were followed by an intensive leaf disease control program, in which all commercial bananas in the affected area were pruned to zero leaf disease (including yellow Sigatoka) and sprayed with fungicides weekly for six months. All unmanaged plants in the area were removed. The treatments did not extend to *Heliconia psittacorum*, which occurs at about the same density as unmanaged bananas in the affected area, because its susceptibility to black Sigatoka (Gasparotto et al 2005) was not suspected at the time.

Black Sigatoka disease was not found in the affected area after November 2001 and the incursion was declared eradicated in October 2005 (Peterson et al 2005). Isolates of *M. fijiensis* from the 2001 Tully incursion were of different genotypes to those found in previous incursions on Cape York Peninsula and thus were considered to be new introductions (Thomas-Hall et al 2005).

Black Sigatoka disease is regarded as having a restricted distribution in Australia and remains under official control.

Biology

Much of the knowledge of *M. fijiensis* relies on knowledge of *M. musicola* and this is reflected in the following review. Additional information is drawn from the literature on related ascomycete fungi, including other *Mycosphaerella* spp., *Venturia inaequalis*, *Monilinia vaccinii-corymbosi* and *Sclerotinia sclerotiorum*.

The black Sigatoka pathogen may be found in four forms:

Mycelial thallus

Mycosphaerella fijiensis has a haploid mycelial thallus (Carlier 2004). On susceptible hosts, mycelial strands are both epiphytic and intercellular. Stomatopodia are formed over stomatal pores on the leaf surface before penetration into the substomatal cavity and intercellular spaces of the mesophyll tissue. These steps are favoured by fluctuating humidities in daylight hours, indicating a hydrotropic response by the fungus to an open stomate (Leach 1946; Goos and Tschirch 1963; Meredith 1970).

A feature of *M. fijiensis* is the extensive growth of epiphytic mycelia in moist environments (Carlier et al 2000). This growth is favoured by water films or near saturated humidity. It is expected that these mycelia will tolerate short periods of low humidity, as is the case with *M. musicola* (Goos and Tschirch 1963; Meredith 1970). Epiphytic mycelia are subject to microbial degradation and antagonism, especially after prolonged exposure (Carlier et al 2000). Leach (1946) considered that antagonistic microflora were responsible for the apparent resistance of old leaves to infection by *M. musicola.*

Anamorph

The anamorph of *M. fijiensis* is a product of asexual reproduction on the mycelial thallus. It involves conidiophores and conidia that are distinctively different from *M. musicola.*

Conidiophores are formed on an inconspicuous stroma and emerge singly or in small groups through stomata within the boundary of leaf lesions. They are 16.5–62.5 × 4–7 µm straight or slightly curved, 0–5 septa and sometimes a basal swelling up to 8 µm in diameter. They are pale to medium greenish brown and paler towards the tip than at the base. The cells on which conidia are formed are up to 25 µm long, 2–4 µm wide at the apex and have 1–4 minutely thickened scars.

Conidiophores first appear at the initial speck stage and new conidiophores continue to be produced at the edge of lesions until the second spot stage (Table 6.1). Most are produced on the lower surface of the leaf at the first spot stage (Meridith 1970; Carlier et al 2000). Meredith and Lawrence (1969) found conidiophores emerged from 5-15% of stomates on the surface of infected areas of Cavendish leaves. There were approximately 13 conidiophores per mm^2 of streak lesions. Fouré and Moreau (1992)

observed up to 44 conidiophores per mm^2 of streak lesions in Cameroon's rainy season but none in the dry season.

Conidia are 30–132 × 2.5–5 µm, 1-10 (commonly 5-7) septate, straight or slightly curved, and truncated at the base with a conspicuous hilum. They are almost colourless to pale green or olivaceous. Conidia are formed singly at the apex of the conidiophore, standing upright at an angle of 45-90º but later becoming lateral as the conidiophore develops (Meredith et al 1973). Up to four mature conidia may be attached to a single conidiophore at a given time.

Conidia are dislodged from conidiophores by both wind and water (Meredith 1973; Aylor 1990; Carlier et al 2000). They can be detected in the air on days without rainfall, especially when traps are set within the banana leaf canopy (Gauhl 1994). Dispersal in dry air is facilitated by the spores being formed on conidiophores raised above the leaf surface, whereas dispersal of prone conidia is more likely to be dependent on water-splash. Whatever the mode of transmission, conidia contaminate any surfaces in the vicinity, including fruit surfaces. Gasparotto et al (2000) found 11,000 conidia on the surface of banana cv Prata Anã (AAA) fruit sampled from plants with severe black Sigatoka symptoms.

With *M. musicola,* conidia germinate within 2-3 hours of deposition on a moist leaf surface at 26-28°C, or over a greater time at sub-optimal temperatures (Meredith 1970). Germination and germ tube development occur only in the presence of free water. However, germ tubes and epiphytic mycelia can withstand short periods of dryness, resuming development when surface moisture is restored.

With *M. fijiensis,* conidia germinate most readily in free water but some germination has been recorded *in vitro* at 92% relative humidity (Jacome et al 1991). Jacome and Schuh (1992) inoculated the under surfaces of banana leaves with conidia produced in culture. About 10^4 conidia with a viability of 90-96% and were inoculated on each marked 84 cm^2 area of banana leaf. The plants were first incubated at 26 ºC in darkness for 18 hours at a minimum of 94% relative humidity, or with periods of 9 hours and 18 hours of leaf wetness superimposed. The plants were then incubated in growth cabinets with a 12-hour photoperiod and minimum of 94% relative humidity at temperatures of 22-31 ºC. Streak symptoms developed 28 days after inoculation when the banana leaves were incubated under a constant high relative humidity. A greater number of lesions developed if the leaves were kept wet for 9 or 18 hours immediately after inoculation, and the incubation time correspondingly reduced to 21 and 14 days. The total number of lesions formed was independent of incubation temperature in the range of 22-28ºC. One lesion developed for every 226-294 viable conidia inoculated onto leaves under the constant 94% relative humidity regime, or for every 129-186 viable conidia inoculated under the wet leaf regimes. The infection efficiencies (lesions produced per viable spore) ranged from 3.40E-03 to 7.76E-03, depending on the pathogen strain and incubation conditions (Figure 6.2). The infection efficiency of conidia inoculated onto the upper surfaces of leaves was not estimated in these experiments but other research (Leach 1946; Pont 1960a; Meredith 1970; Carlier et al 2000) indicates that infection is much less common.

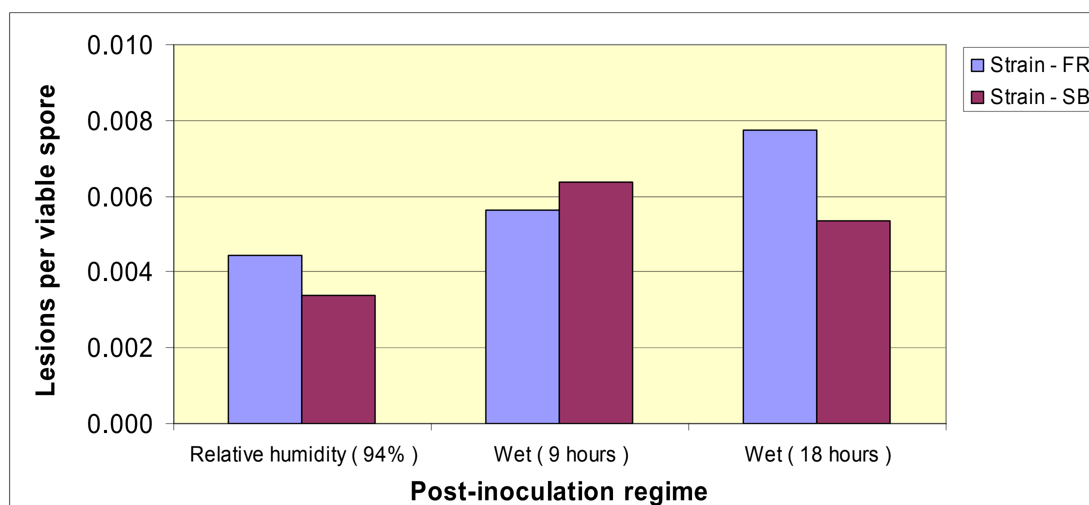


Figure 6.2 Infection efficiency of *M. fijiensis* conidia
Source: from Jacome and Schuh (1992)

Gasparotto et al (2000) reported that conidia of *M. fijiensis* remained viable for at least 60 days on detached banana leaves, for up to 30 days on artificially contaminated cardboard, wood, plastic and tyres, up to 18 days on fruit peel (until it rotted) and for up to 10 days on iron. Gasparotto et al (2000) also reported that benomyl, ecolife and quaternary ammonia compound at 100 ppm totally inhibited the germination of conidia *in vitro.* They also found that a post-harvest dip of banana fruit for five minutes in 100 ppm or 200 ppm concentrations of these fungicides totally inhibited the germination of conidia.

Fungicides used in disease control programs in both the Philippines and Australia inhibit the production of conidiophores and conidia and also the germination of conidia (Stover 1972; Carlier et al 2000). Water splashed from leaves treated with fungicides also inhibits the germination of spores on young, untreated leaves lower in the canopy (Meredith 1973).

Andromorph

Spermogonia develop in substomatal cavities at the stage when leaf streaks develop into spots. They are more abundant on the lower surface of the leaf, being consistently associated with the stromatic tissue on which conidiophores are also produced (Meredith and Lawrence 1969). Spermogonia are 55–88 × 35–50 µm and they eventually protrude through the stomatal pore. Spermatia are 2.5–5.0 × 1.0–2.5 µm, rod-shaped and hyaline and they are released in the presence of free water. They are thought to function as male gametes in sexual reproduction (see discussion on sexuality below).

Teleomorph

The teleomorph of *M. fijiensis* is a product of sexual reproduction. It involves pseudothecia and ascospores that are morphologically indistinguishable from *M. musicola*. Even though these species are genetically distinct (Carlier et al 2000), much of the literature on teleomorph biology is applicable to both species.

Pseudothecia are formed within the diseased tissue but possess an ostiole that breaks through the epidermis at maturity. When mature, pseudothecia are dark brown, thick-walled, generally globose and 47−85 µm in diameter.

Each pseudothecium has 10–27 asci (Stover 1972) and each ascus has eight ascospores measuring 11.5–16.5 x 2.5–5.0 µm. A pseudothecium may therefore produce a total of 80–216 ascospores.

Pseudothecia first appear at the second spot stage (Table 6.1). They may be found on the upper and lower leaf surfaces of leaves that have not been sprayed with fungicides at average densities of 0.3-1.2 and 0.6-3.7 per mm^2 of lesion, respectively (Burt et al 1999; Carlier et al 2000). Fungicides used in the control of black Sigatoka, such as propiconazole (Stover and Dickson 1985) and mancozeb and chlorothalonil (Guzmán and Romero 1998), reduce the density of pseudothecia in infected leaf tissue.

Dry weather and cool temperatures inhibit production of pseudothecia, while cool temperatures delay their maturation (Stover 1964; Gauhl 1994). There is evidence that pseudothecia continue to mature in necrotic tissue when that tissue is removed from the plant (Gauhl 1994; Burt et al 1999), but no reports have been found on the development of pseudothecia in black Sigatoka lesions that are removed from the host plant before reaching the second spot stage. The occurrence of pseudothecia in fruit skin tissue has not been reported but seems unlikely given that the development of symptoms is restricted.

Mature asci begin to release their ascospores within 10 minutes of being thoroughly wetted (Stover 1964; Carlier et al 2000). The release process involves a violent discharge and is independent of temperature and other climatic factors (Meredith et al 1973; Gauhl 1994). Although not measured for *M. fijiensis,* the extent of discharge in *Guignardia citricarpa* is about 10 mm (Kiely 1949) and 1-7 mm in *Venturia inaequalis* (Aylor and Anagnostakis 1991). Once released, the ascospores are subject to gravitational forces resulting in sedimentation at a rate of about 2 mm/s in still air. However, they are affected to a much greater extent by air convection currents that may transport them at speeds of 200 mm/s or more (Aylor 1990).

Large numbers of ascospores can be detected in the air on days with intermittent rain or heavy morning dews (Meredith et al 1973; Gauhl 1994), both within and above the leaf canopy of diseased banana plants (Gauhl 1994). Their numbers are greatest when rain occurs after a few days without rain, declining during extended periods of rainfall and particularly during periods of heavy rain (Meredith et al 1973). It is believed that ascospores can contaminate any surfaces in the areas to which they are dispersed but no information has been found on the degree of contamination of fruit surfaces like that reported for conidia above.

Stover (1964) considered that pseudothecia of *M. musicola* became infertile once they had discharged their ascospores. He noted that subsequent wetting and drying resulted in the discharge of few or no ascospores. Similarly, pseudothecium-bearing, dry, mass-infected tissue collected in the field within two days after rain did not contain many mature ascospores. Stover (1971) noted that prolonged storage of pseudothecia at high relative humidity led to spontaneous release of ascospores and consequent infertility within 10 weeks of storage. Half the leaf samples failed to discharge ascospores after 8 weeks even at low humidity in the day and high humidity at night, as might occur when infected leaves are exposed on the ground.

Gauhl (1994) found that ascospores were released from black Sigatoka lesions at least 21 weeks after their formation when the lesions were sampled from leaves left hanging in the plantation. When black Sigatoka lesions aged one week were placed on the ground, large quantities of ascospores were released from lesions at ages 2 and 3 weeks but only small quantities at ages 6 and 8 weeks. The leaf tissue had rotted by the time the lesions were aged 10 weeks and no ascospores could be recovered. Similar observations were made on *M. musicola* by Peterson et al (1998) with respect both to leaves left hanging on plants and those laid on the ground. Peterson et al (2003) ensured that leaves bearing pseudothecia were removed from banana plants during the eradication campaign at Tully, thereby ensuring that the pseudothecia survived for no longer than 4-8 weeks instead of persisting for >21 weeks if left on the plant.

Burt et al (1999) estimated that 4.5 ascospores were ejected into the air, on average, from each *M. fijiensis* pseudothecium. This represents only 2–6% of the ascospores potentially present. The experiment was carried out with repeated wetting and drying until no further ascospores were released. The reasons given for this low recovery related to uncertainty about the maturity of pseudothecia used in the experiments. A greater number of ascospores were released from class 3 leaves (16-33% necrotic tissue) than from class 5 leaves (>50% necrosis). Also, a greater number of ascospores were

released from class 3 leaves on the second cycle of wetting than on the first. This indicates that pseudothecia continued to mature during the course of the experiment, especially on class 3 leaves. It also indicates that some pseudothecia had already released their ascospores prior to the experiment, especially on class 5 leaves. Burt et al (1999) also thought that the poor recovery may have been related to the failure of many ejected spores to break free of the boundary layer on the leaf surface. Burt et al (1999) did not provide information on the condition of the leaf material prior to experimentation. However, Kiely (1949) observed that the extent of violent discharge from *G. citricarpa* asci decreased after the initial release period and considered that storage and incubation conditions affected the efficiency with which ascospores became air-borne. Aylor and Anagnostakis (1991) also observed that ascospore ejection decreased after the initial release and considered that it was related to a decrease of hydrostatic pressure in the asci. They also observed that ascospores were obstructed by water films on the leaf surface.

Aylor and Qiu (1996) examined the release of *V. inaequalis* ascospores from a ground-level source in which the potential ascospore dose was estimated to be 1.50E+08 per m^2. The infected leaf litter was in a grass sward that was 25 cm tall at the start of the release period and 60 cm at the end. They estimated that about 5% of the ascospores were deposited on the short grass and about 20% on the tall grass. The remaining ascospores became airborne and were concentrated mainly within one metre of the sward surface. The release occurred over a period of 60 days but the rate of release declined as the release period progressed. Ascospores settle on any surface in the vicinity as a result of direct impact or of becoming involved with falling water droplets (Aylor 1990; Aylor and Sutton 1992).

Ascospores germinate within 2-3 hours of deposition on a moist leaf surface at 26-28 °C, or over a greater time at sub-optimal temperatures (Carlier et al 2000). Research on *M. musicola* indicates that germination and germ tube development occur only in the presence of free water (Stover 1972; Meredith 1970; Carlier et al 2000). Germ tubes and epiphytic mycelia have been shown to withstand short periods of dryness, resuming development when surface moisture is restored (Meredith 1970). With *M. fijiensis,* some germination has been recorded *in vitro* at a relative humidity of 98% (Jacome et al 1991) but infection did not occur unless the leaves were wet (Jacome and Schuh 1992). This appears in conflict with evidence for *M. musicola,* where fluctuating humidity in daylight hours favours penetration through open stomates (Goos and Tschirch 1963; Meredith 1970).

No information has been found on the overall infection efficiency of *M. fijiensis* ascospores but Sanogo and Aylor (1997), in studying *V. inaequalis* ascospores, report values of 6-21% on young apple leaves.

Stover (1971) reported that ascospores of *M. musicola* failed to germinate if released from pseudothecia that had been stored for 4 weeks in the sun at 15-38 ºC, for five weeks on a laboratory shelf at 22-28 ºC, or for 8 weeks if stored in the shade at 15-35 ºC. However, they retained their viability for at least 10 weeks if stored in a refrigerator at 5-7 ºC.

Once released, ascospores are subject to adverse effects of desiccation and radiation in a manner similar to conidia. However, little information has been found on the sensitivity of *M. fijiensis* ascospores to adverse environments. Parnell et al (1998) reported that ascospores can withstand ultraviolet radiation for up to six hours. Whiteside (1974) found that ascospores of *M citri* (the causal agent of greasy spot on citrus) are not long-lived if conditions are unfavourable for germination and infection.

Fungicides used in disease control programs in both the Philippines and Australia inhibit the production of pseudothecia and ascospores and also the germination of ascospores (Stover 1972; Carlier et al 2000). Guzmán and Romero (1998) report that mancozeb reduced the production of ascospores by 74% and chlorothalonil by 42%. Water splashed from leaves treated with fungicides also inhibits the germination of conidia and ascospores (Leach 1946; Meredith 1973).

Sexuality

Mycosphaerella fijiensis is heterothallic, in that compatible mating types are required for sexual recombination (Mourichon and Zapater 1990; Etebu et al 2003). Cultures produced from single ascospores or conidia are sterile in that they do not form pseudothecia. It is thought that spermatia produced in spermogonia move to specialised mycelia (trichogynes) produced in the early stages of pseudothecial development (protopseudothecia) and initiate cross-fertilization between compatible mating types (Carlier et al 2000). Pseudothecia with ascospores are found in culture about 35 days after fertilization (Etebu et al 2003). Carlier (2004) notes that a stable gametic equilibrium is maintained through random mating at the plantation and plant scales. In *M. citri,* segregation for mating types occurs in a 1:1 ratio within individual asci (Mondal et al 2004).

Effects of moisture

The presence of water plays a key role in four aspects of the epidemiology of black Sigatoka. The release and dissemination of spermatia is discussed under the heading *Sexuality* above. The release of ascospores is also discussed under the heading *Teleomorph* above. The third aspect concerns the dissemination of spores by rain splash, and this is discussed under the heading of *Carriage in water* below.

The fourth aspect concerns spore germination and the growth of germ tubes and epiphytic mycelia (Jacome et al 1991; Carlier et al 2000).

Ascospores and conidia germinate most readily in free water. Under laboratory conditions, ascospores require 98% relative humidity to germinate but conidia can germinate to some extent at a relative humidity of 92% (Jacome et al 1991). In the field, the pre-penetration period can extend over several days of wet and dry conditions. This may involve intermittent rainfall or dew (Meredith 1970).

The Australian Bureau of Meteorology provides data on the number of days per year when rainfall exceeds various amounts per day (see Figure 2.4 of Part C). In populated areas of Australia where banana waste is generated, there may be an average of 70-100 days per year when more than 1 mm of rain occurs. However, some dew could be expected on most days of the year and therefore surface moisture is available for at least some germination and growth of *M. fijiensis* throughout the year.

Stover (1968) considered leaf wetness to be a factor for infection by *M. musicola* in Honduras and Allen et al (1992) collected leaf wetness data for a number of sites in banana growing areas of Australia. The data from Modanville, New South Wales, and South Johnstone, North Queensland, has been re-analysed (Allen 2008) and the results are summarised as follows. At Modanville, only six days of 354 days counted had a total of more than 18 hours of leaf wetness over the day. There were no periods when wetness continued for the full 24 hours. About 80% of hourly periods were dry and surface wetness persisted for less than six hours after wetting. At South Johnstone, there were 20 days of 330 days counted with more than 18 hours leaf wetness per day but no days were wet for a full 24 hours. About 30% of hourly periods were dry but surface wetness persisted for less than seven hours after wetting. There was a correlation between monthly averages of rainfall and surface wetness, indicating that 1 mm of rainfall led to 1-3 hours of wetness in the day and 3-5 hours at night (including an average of two hours of dew). It is noted that the years 1991 and 1992 were drier than average so the number of days of rainfall and wetness may be lower than normal, but the correlation between surface wetness and rainfall should still hold.

Effects of temperature

Temperature affects all aspects of the growth and development of *M. fijiensis* (Carlier et al 2000). This includes the growth of mycelium, the production of conidia, the initiation and maturation of pseudothecia, and the production of ascospores. Leach (1946) determined that the minimum temperature for vigorous growth of *M. musicola* was about 18 ºC but noted that some germination occurred at 17 ºC. Carlier et al (2000) report that the minimum temperature for development of

M. fijiensis is 12 ºC and the maximum is 36 ºC. Allen et al (1992) examined available experimental evidence and determined that the optimum temperature for growth and development of *Mycosphaerella* spp. on bananas was about 27 °C and that the relative growth rate ($RGR_{temperature}$ decreased steeply at sub–optimal temperatures. At 21.5 °C it is 50% of that at the optimum temperature (Figure 6.3).

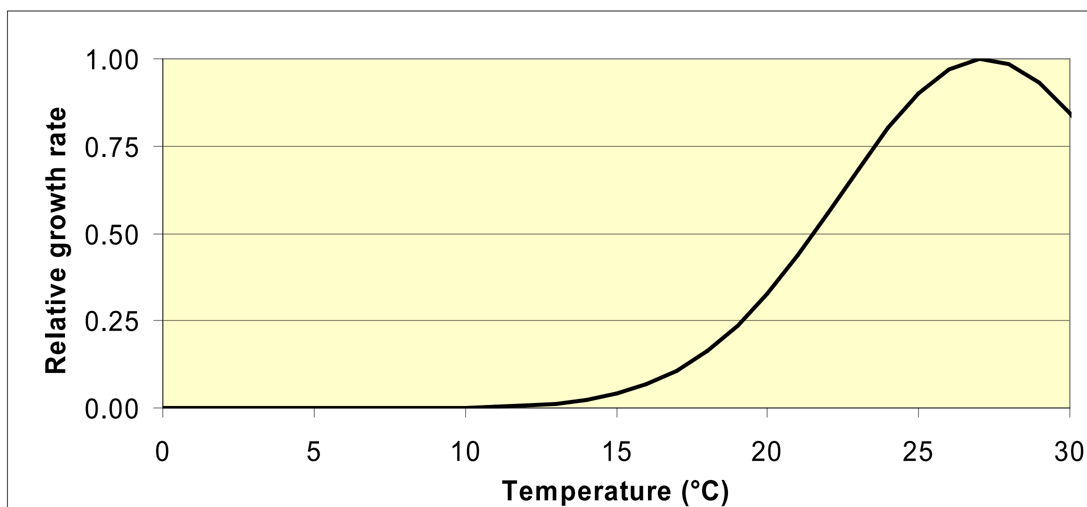


Figure 6.3 Effects of temperature on the relative growth rate of *Mycosphaerella* spp.
Source: from Allen et al (1992)

The maturation of pseudothecia, the production of conidia, and the germination of both conidia and ascospores correlate with the average daily temperature. Average monthly temperatures for selected locations in the Philippines and Australia are presented in Figure 2.1 and Figure 2.2, while the relative growth rates estimated for these locations (Allen et al 1992) are presented in Figure 6.3.

At Davao City in the Philippines, temperatures are near optimal for *M. fijiensis* in all months of the year. The average relative growth rate of *M. fijiensis* in Davao City is approximately 1.0 (Figure 6.4). In Australia temperatures are often sub–optimal (see the temperatures for Sydney and Melbourne) and average relative growth rates vary depending on location and month of the year. Most of these locations have unfavourably low temperatures, but Kununurra is exceptional in that it has unfavourably high temperatures in the summer months.

When averaged over the entire year, the relative growth rate is 0.5 in grower areas and 0.2 in other areas, ranging from 0.3–1.0 and 0–0.3 depending on location. The number of months when the relative growth rate is less than 0.5 is closely correlated with these estimates.

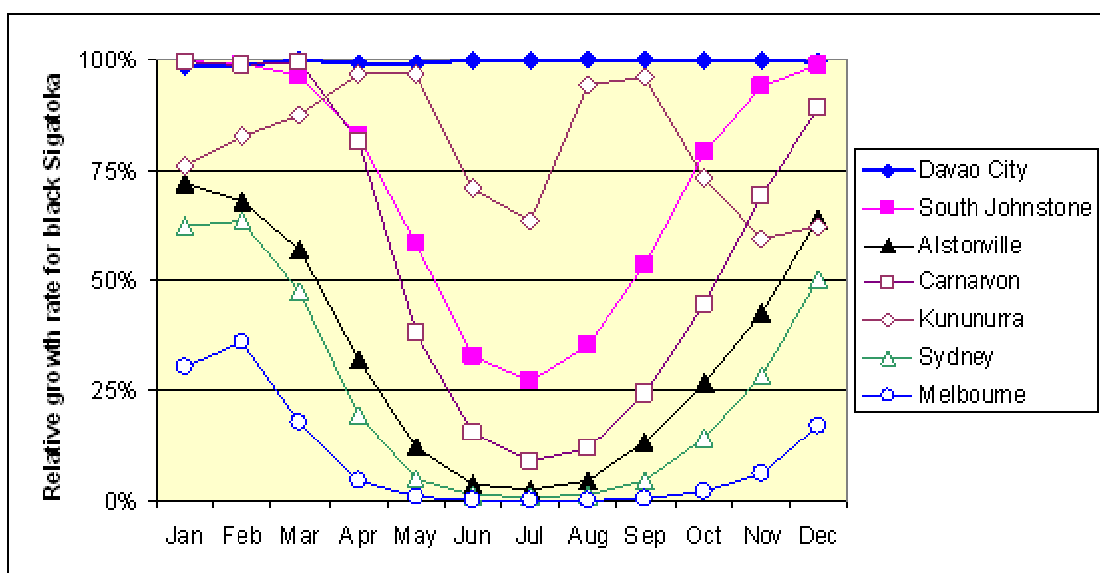


Figure 6.4 Monthly relative growth rates of *Mycosphaerella* spp.

Source: from Allen et al (1992)

Interactions between temperature and moisture

Under optimal conditions of temperature and moisture, black Sigatoka spores germinate within 2–3 hours of deposition. Penetration of host tissue occurs after 2–3 day's growth of the epiphytic mycelia (Carlier et al 2000). Further epiphytic growth and penetration occurs over the following 2–3 weeks before symptoms appear and sporulation occurs. The processes also occur under sub-optimal conditions but at a slower rate (Jacome and Schuh 1992). The degrees of line and tip spotting reflecting the abundance of conidia and ascospores of *M. musicola*, respectively, follow a seasonal pattern with the greatest intensity at the end of the tropical wet season (Leach 1946; Pont 1960a).

Infection events can be described by arbitrary rules such as the occurrence of three consecutive wet days (>1mm rain) with a minimum temperature greater than 18 ºC. Peterson et al (2005) reported an average of 1–3 infection events per month in the years 2001-2005 at Tully (North Queensland). These criteria were applied to meteorological data from South Johnstone (representing exposure groups in north Queensland), Alstonville (representing exposure groups in south-eastern Queensland and north-eastern New South Wales), Sydney (representing exposure groups in the northern parts of other areas of Australia) and Melbourne (representing the southern parts of other areas). The data were recorded over the 11 years from 1996 to 2006, inclusive. Table 6.3 shows there was an average of 79.5 infection events per year at South Johnstone (range 56–110 between years), 9.3 at Alstonville (range 3–18) and 2.5 at Sydney (range 1–4). There were no infection days at Melbourne for the period analysed.

Table 6.3 Annual infection events for banana black Sigatoka

Source: based in criteria proposed by Peterson et al (2005)

Year	Melbourne	Sydney	Alstonville	South Johnstone
1996	0	2	10	81
1997	0	4	10	59
1998	0	1	7	95
1999	0	4	18	87
2000	0	1	3	110
2001	0	2	9	62
2002	0	4	5	56
2003	0	2	15	56
2004	0	2	10	87
2005	0	2	5	69
2006	0	3	10	113
Average	**0**	**2.5**	**9.3**	**79.5**

Infection events can also be described by a cumulative infection index, involving the measurement of $RGR_{temperature}$ (Figure 6.3) at times when leaves were wet and then adding the values on a daily or relevant growth period basis. Allen et al (1992) found that the cumulative infection index over the period between bunch emergence and harvest correlated well with the severity of mixed *M. musicola* and *M. musae* infections assessed by the number of functional leaves remaining on bunched stems at harvest. This index has been used as a guide for fungicide spray scheduling in New South Wales since 1989.

Dispersal

Carriage on plant material

The pathogen can be carried on plant material (such as leaf material used to pad bunches and fruit in transit or attached to nursery stock) and this provides an ideal means of transferring mycelial thalli and pseudothecia, from which spores may subsequently disperse (Stover 1972; Carlier et al 2000). Under North Queensland conditions, it was considered that the human-assisted movement of plant material could have spread black Sigatoka over a distance of up to 50 km before it was detected at Tully in 2001, hence the initial restriction zone for fruit marketing.

Fullerton (2006) reported that there were endophytic mycelial thalli in the skin of banana fruit and that there was some evidence also of stromatic tissue like that formed by *M. fijiensis* on leaves. Gasparotto et al (2000) observed that fruit became contaminated with conidia in the field and it is expected that fruit would be contaminated with ascospores similarly. Peterson (2001) speculated that the introduction of black Sigatoka to Jamaica was most likely with or on fruit from Costa Rica. However, it is not known if these fruit were packed in cartons essentially free of leaf material, as they are in Australia and the Philippines, or whether they were padded with banana leaves as traditionally practiced in the Caribbean and elsewhere (Wardlaw 1961; Stover 1972; Gasparotto et al 2000).

In December 2005, 351 cartons of Philippine bananas were examined at a wholesale centre in Christchurch, New Zealand (Peterson et al 2006). No leaf material was found in 30 cartons from one supplier, but 16 pieces of leaf material were found among 321 cartons examined from a second supplier. One carton had six pieces of leaf material, one had two pieces, and seven cartons had one piece each. The pieces of leaf material varied in size (1–3 mm × 2–25 mm) and were generally found between the fingers of the packed clusters of fruit. A later study (ABGC 2006b) found *M. fijiensis* to be present in four of eleven leaf fragment samples tested.

A total of 3597 clusters were packed in the cartons from the second supplier, indicating an average of 0.004 leaf pieces per cluster. Assuming that counts per cluster follow a Poisson distribution, the

likelihood that a cluster would have at least one piece of leaf material at the time of arrival at a retail outlet is 0.004. No information has been found on how this estimate might vary between suppliers and at different times of the year.

The study of Peterson et al (2006) also found floral remnants in 148 of the 351 cartons examined. These remnants were found attached to the fruit and also within the polythene liner of packed cartons. A couple of floral bits were found wedged between fingers near the crown of the cluster. Peterson et al (2006) did not report on whether or not the leaf or floral fragments were fresh or necrotic, or whether or not they contained pseudothecia.

Carriage in air

It is expected that ascospores and conidia of *M. fijiensis* can be dispersed over considerable distances by convective air currents. This is illustrated by the following examples.

- Meredith et al (1973) detected ascospores one kilometre from a presumed source of infection. In this study, disinfected corms of Gros Michel banana were planted downwind of a diseased Gros Michel plantation (the only diseased bananas in the vicinity). The corms produced young shoots and, within two months, the lower leaves had numerous black Sigatoka lesions. Examination of the younger leaves showed numerous germinated ascospores of black Sigatoka on the surfaces, particularly the lower surface. Ascospores were also trapped in that location using a Hirst spore trap and identities were confirmed by transferring single spores from the trap slides to agar and allowing cultures to develop.
- Gauhl (1994) and Burt et al (1999) detected ascospores and conidia in spore traps in the vicinity of infected banana plantations. They considered that ascospores may be transported at least five kilometres and possibly over 40 kilometres. Burt et al (1997) detected conidia at a height of three metres, about one metre above the leaf canopy. There was a positive correlation between the number of conidia detected and wind speed, demonstrating that conidia as well as ascospores are wind-borne.
- Mondal et al (2003) indicate that large numbers of *M. citri* ascospores spread horizontally and vertically from infected citrus leaves lying on the ground, reaching a height of at least 7.5 metres and a distance of at least 90 metres downwind of the source.

There is a body of local evidence in Australia that suggests that spread of black Sigatoka occurs over large distances from sources with low inoculum levels.

- <u>Infected properties in the outbreak at Tully North Queensland (ABGC 2007).</u>

  On the plantation on which the incidence and intensity of disease symptoms indicated was the original site of infection, the spread from plants with the most severe symptoms (being a clump of plants of about 12 stems behind a building) ranged from 30 metres to the west to 250 metres to the east and 300 metres to the south east. Sugarcane was growing between the originally infected plants and the other blocks of bananas. The distances between the 25 infested sites (13 managed commercial blocks and 12 non-managed blocks of plants) were: 3 sites <1 km apart, 11 sites 1-2 km apart; 7 sites 2-5km apart; 4 sites >5km apart (1 x 7km, 2 x 8km, and 1 x 16km). However, much of the evidence for secondary spread was destroyed as a result of diseased leaves being removed during the response program. The possibility of other vectors being involved, such as the carriage of plant material, cannot be ruled out.

- <u>Market access restrictions on fruit produced near black Sigatoka infected plants (Allen 2001).</u>

  Following the initial detection of black Sigatoka near Tully in 2001, restrictions were imposed on the marketing of banana fruit produced within 50 km of infected properties. This restriction was based on considerations of the carriage of black Sigatoka in plant material and in air. Bananas from within this zone could not be marketed in the rest of Queensland, or anywhere in New South Wales or Western Australia. The restricted zone was reduced to a radius of 7.5 km after Queensland authorities had completed initial delimiting surveys and had also introduced effective

controls on the movement of plant material and the treatment of diseased plants. Bananas produced more than 7.5 km from an infected property could then be marketed in Brisbane and Sydney. Queensland authorities submitted firstly that inoculum levels had been reduced by treatment and secondly that the mean distance of spread in air was about 200 metres for ascospores and five metres for conidia. The assumptions used in this second assessment were based on general evidence on the dispersion of small organisms (Wolfenbarger 1959; Gregory 1968). The sedimentation rate for the large conidia was considered to be greater than for the smaller ascospores. Market access to grower areas in New South Wales and Western Australia was not restored until after black Sigatoka had been declared eradicated from the Tully area in October 2005.

- Yellow Sigatoka on sentinel plants (Peterson et al 2003).

  During the program developed to demonstrate area freedom from black Sigatoka after the eradication campaign in the Tully Valley, yellow Sigatoka became established on susceptible sentinel plants at least one to two kilometres from plantations or non-commercial banana sites. This could indicate that yellow Sigatoka spread from an extremely low level of inoculum because yellow Sigatoka had been controlled to almost zero disease levels in commercial plantations during the eradication program as a result of regular de-leafing and fungicide spraying. However, the occurrence of yellow Sigatoka on the sentinel plants could also be explained by spread from vast quantities of yellow Sigatoka inoculum in neighbouring areas to the north and south of Tully.

- Statistical models of disease spread (Jorgensen et al 2004).

  As part of the process to demonstrate area freedom following the black Sigatoka outbreak in Tully, models were developed to predict the likelihood of spread and establishment from undetected remnants of the black Sigatoka population. It was noted that the rate of deposition of spores that spread by the wind over a long distance depends on the weather conditions at the time. For the model, an average value was assumed that corresponded to about half of the spores being deposited out of the air by two kilometres and most of the spores being deposited by 20 kilometres. The model recognised that long distance spread could occur from a single remnant source. It predicted, for example, that 0.2 lesions would have developed on susceptible host plants 5-10 km from the source when only 9 lesions were present at the source.

In general, the inoculum in the above examples was released from an unspecified quantity of infected tissue over an extended period of time. In most cases, the inoculum was present in leaves hanging on the plants and dispersal gradients were measured at a considerable distance from the source (that is, in the tail of the distribution). This represents a scenario quite different from that of a small amount of inoculum associated with banana waste that has been discarded on the ground. No information has been found on the dispersal of *M. fijiensis* ascospores or conidia from a small quantity of waste material such as the skin of banana fruit or the fragments of associated leaf and floral tissue. However, four studies have some relevance to the situation with banana waste on the ground:

- Carlier (2004) studied dispersal of *M. fijiensis* around an inoculum source over a period of about one month. This indicated a negative exponential gradient and an estimated dispersal distance of around 30 metres.
- Cox and Scherm (2001) studied primary infections of blueberry plants caused by ascospores of *Monilinia vaccinii-corymbosi.* The inoculum was in the form of 30 pseudosclerotia buried at a point on the upwind side of a blueberry field. These pseudothecia produced apothecia and released ascospores over a period of two weeks. Primary infections developed as blighted shoot tips over eight week periods in the 1999 and 2000 seasons. In 1999, more than 95% of primary infections occurred within 20 m of the source and all observed infections within 40 m of the source. In 2000, about 95% of infections occurred again within 20 m of the source and all within 30 m.
- Bourdôt et al (2001) studied dispersal of *Sclerotinia sclerotiorum* ascospores from inoculum sources on the soil beneath pastures of varying sward densities. This indicated a negative

exponential gradient and an estimated dispersal distance of 2.5-7.9 m downwind before the deposition was equivalent to background levels. A later analysis (de Jong et al 2002) showed that varying proportions of ascospores released from apothecia on the ground entered turbulent air above the pasture sward, depending on the depth of the pasture sward and the air speed or turbulence. It was estimated that only 5% of ascospores escaped vertically from a dense sward, even in wind speeds of 7 m/s, whereas up to 50% of ascospores escaped from open swards. The ascospores that entered the turbulent air were apparently dispersed over considerable distances.

- Aylor and Qiu (1996) found that a large proportion of *V. inaequalis* ascospores were released into the air from infected apple leaf litter amongst a grass sward on the ground. The leaf litter had a potential of about 1.5 x 10^8 ascospores per m^2 of ground surface. About 5% of the ascospores deposited on the grass sward when 25 cm tall and up to 20% were deposited when the sward was 60 cm tall. The released ascospores became increasingly involved with convective air currents as they rose above the sward. They were detected at least 3 m above the sward but at a much lower concentration than within 1 m of the sward. Horizontal fluxes of ascospores were in the order of 10,000-60,000 spores $m^{-2}s^{-1}$ within 1 m of the ground surface compared with 0–5,000 spores $m^{-2}s^{-1}$ at 3 m.

Carriage in water

While dispersal of *M. fijiensis* ascospores and conidia is considered to be mainly air-borne (Carlier et al 2000), both types of spores can be splash-dispersed or carried in water droplets (Stover 1972; Carlier et al 2000). Line spotting symptoms occur when *M. musicola* conidia accumulate in the waterfilled spaces between unfurled banana leaves (Leach 1946; Stover 1972). These symptoms are more prevalent on the leaves of developing suckers that receive run-off water from above than on the leaves of the tallest plants.

In the case of dispersal by rain-splash, Meredith (1973) described early research reporting that a large raindrop 5 mm in diameter (about 65 µl) falling vertically onto a wet surface can disperse more than 5,000 droplets to a maximum distance of 100 cm and raise them to a height of 40 cm. About half of the larger reflected droplets (mean diameter of 150 µm) were found to carry spores of *Fusarium solani* to a mean distance of 20 cm without becoming truly airborne. Such large drops rarely fall directly as rain but occur when run-off water drips from leaves during rainy weather. A feature of rain-splash is that spores can be re-splashed one or more times. The smallest reflected droplets may form aerosols and later the spores become truly airborne as the water evaporates.

More recent studies using rainfall simulators (Fitt et al 1989; Yang et al 1990; Huber et al 2006) have found that ground cover, plant canopy density and rain intensity affect the extent of splash dispersal. In still air, dispersal of *Colletrichum acutatum* to strawberry fruit was greatest from smooth horizontal surfaces, extending up to 120 cm, but the distance from soil or straw was considerably reduced. Similar results have been reported with *Pseudocercosporella herpotrichoides* conidia in wheat and wheat-clover cropping systems (Soleimani et al 1996). In wind tunnel experiments, conidia of *P. herpotrichoides* have been detected in water droplets up to 10 m downwind of the source (Fitt et al 1989). These conidia are similar in size to those of *M. fijiensis.*

There are physical limitations on the inclusion of fungal spores in small water droplets (Huber et al 1989). Spores as large as *M. fijiensis* conidia are rarely found in droplets <100 µm in diameter. Ascospores may be found in smaller droplets, which are expected to disperse further in wind than the larger droplets. The dispersal of small *Colletotrichum gloeosporiodies* conidia has been reported to have half-distances of 1-10 m in the field (Huber et al 2006).

It is expected that the degree of splash-dispersal will be directly proportional to the amount of rainfall or irrigation received, and indirectly to the frequency of rainfall. Spores dispersed from the upper canopy will travel primarily in a downward direction, although wind speed and direction will influence the extent of horizontal dispersal to some extent. Huber et al (2006) provide evidence that the proportion of rainfall splashed from wet surfaces varies with the type of leaf surface, the intensity

of rain and rain droplet size. With tobacco leaves, about 0.1% of incident rain splashed when falling at 10 mm per hour as small (2mm diameter) drops and about 10% with large (4mm diameter) drops. With oilseed rape leaves, the corresponding proportions were about 3% and 20%. The proportion of water splashed was about 10-times lower when rain fell at 1 mm/hr but not significantly greater when rain fell at 100 mm per hour.

Carriage on other vectors

Gasparotto et al (2000) provide evidence that conidia can survive on inert surfaces such as cardboard, metal and timber for a considerable time and that such contamination may be associated with spread of *M. fijiensis* over long distances.

No data have been discovered to substantiate the possibility that animal vectors could spread *M. fijiensis*. Some insects such as shore flies, fungus gnats and moth flies have been found to ingest *Fusarium avenaceum* and *Thielaviopsis basicola* spores and transmit them in their frass (Stranghellini et al 1999; El-Hamalawi and Stranghellini 2005). However, it is not known if these insects would be attracted to banana waste. ABGC (2007) lists *Apis mellifera*, *Atherigona orientalis, Drosophila* spp*.,* *Silba* spp.. *Telostylinus linolatus* and various beetles as being attracted to banana fruit waste. However, no information has been found on the attraction of these insects to susceptible leaf tissue.

Strains of *Mycosphaerella fijiensis*

It is considered that *M. fijiensis* has co-evolved with its *Musa* hosts in South-East Asia, which is also the area of greatest diversity of genes for virulence, resistance and susceptibility (Carlier et al 2002). Founder events associated with the introduction of hosts and the pathogen to other areas led to a reduction in diversity. However, this diversity has been maintained by a process of random mating.

Molecular studies of isolates of *M. fijiensis* from Asia, Australia, Papua New Guinea and the South Pacific have identified five clades. Most of these clades have a wide geographic distribution; clades 2 and 5 for example have been isolated from *Musa* in Australia and the Philippines (Thomas-Hall et al 2005).

The widespread use of fungicides for disease control has led toselection of strains of *M. fijiensis* with fungicide resistance (Carlier et al 2000). Peterson et al (2003) report significant shifts in resistance in Australian populations of *M. musicola* to benomyl and propiconazole as a result of continued use over many years, and evidence for cross resistance between propiconazole and tebuconazole. Fungicide resistance problems have also been encountered in the Philippines (BPI 2002b). Management strategies have been developed to counter fungicide resistance problems (Carlier et al 2000) and are used in both Australia (Peterson et al 2003) and the Philippines (BPI 2002b).

Diagnostic technologies

A preliminary diagnosis of black Sigatoka can be made on the basis of symptoms (Table 6.1). In most cases, confirmation is required using microscopy and molecular biology. Microscopy involves examination of the anamorph for characteristic scars on the conidiophores and thickening of the hilum region in conidia (Carlier et al 2000). Molecular techniques involve amplification of DNA using specific primers (Johanson and Jeger 1993; Johanson et al 1994; Peterson et al 2003). These tests have a reasonable specificity for *M. fijiensis* but a poorly defined sensitivity.

Disease severity in the Philippines

Data provided by Philippines authorities (BPI 2002b) indicated that black Sigatoka disease is found in all Cavendish plantations. The data also indicated that the severity of disease, as measured by the youngest leaf with initial spots on unbunched pseudostems, varied from 8.33 in July 1999 (most severe) to 15.08 in December 2001 (Figure 6.35). It is evident that the disease was present on the leaves all year round and, except for a period of poor disease control in 1999, the disease is controlled

to the extent that initial leaf spots generally appear on leaves 10, 11 or 12. The term “initial leaf spot” was not defined by BPI (2002b) but, if it is assumed that it corresponds to the first spot stage of symptom development in Table 6.1, it is expected that first symptoms of infection (Stage 1) would occur on leaves 2–3 positions above (Gauhl 1994). On bunched pseudostems, first disease symptoms may be present on leaves 4, 5 or 6 because no new leaves are produced on that pseudostem after flowering (Gauhl 1994). However, it is unlikely that all leaves on bunched stems would be affected by black Sigatoka at the time of harvest if routine disease control measures are maintained.

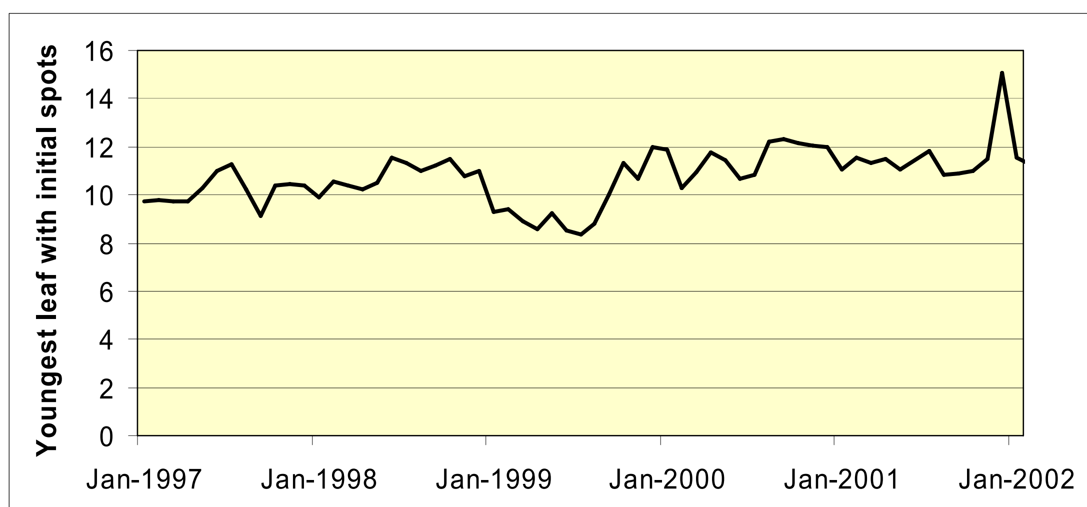


Figure 6.5 Average youngest leaf with initial spot symptoms of black Sigatoka

Based on data provided by Philippines authorities (BPI 2002b) for unbunched pseudostems in export Cavendish banana plantations on Mindanao Island

Impact of black Sigatoka on the banana industry

Black Sigatoka is considered one of the most damaging diseases of commercial *Musa* crops because the destruction of banana leaf tissue by the disease depresses yield, reduces fruit size and leads to premature ripening of fruit (Carlier et al 2000). Black Sigatoka does not kill plants but, after its first occurrence in an area, the disease builds up and often reaches epidemic proportions in a few years. There can be substantial reductions in yield if the disease is not controlled, of the order of 20–50% (Carlier et al 2000). In North Queensland, where 15–25 cycles of fungicides are used for control of *M. musicola*, it is estimated that an additional 10–15 spray cycles would be needed to control *M. fijiensis* if it were introduced to that area (Allen 2000). This could also increase the stringency required of fungicide resistance management strategies. The number of spray cycles in subtropical areas could also increase from the 4–6 cycles per year used for control of *M. musicola* and *M. musae* (Allen et al 1992). In both Australian banana growing areas, the costs of disease control would almost double.

If black Sigatoka was found again on mainland Australia, it is likely that there would be an eradication program such as that undertaken in North Queensland in 2001–05. This program was estimated to have increased the cost of production for Cavendish bananas in the Tully region by $2 per carton. Eradication and surveillance cost over $20 million. In addition, market access restrictions temporarily disrupted the orderly marketing of bananas throughout Australia, with losses amounting to several times the direct costs of the emergency response (C Adriaansen, General Manager, Plant Biosecurity, QDPIF, pers comm 22 March 2006).

In the Philippines, management of Sigatoka diseases and freckle is by application of between 30 to 45 fungicide sprays during the year and the regular manual removal of diseased leaves. Disease lesions are trimmed from leaves that show less than 50% spotting or streaking and whole leaves are removed when 75% shows disease symptoms (PCARRD 1988). Partial removal of infected or damaged leaf

ends and sheaths, and moderate removal of mature and senescent leaves, reduces the severity of black Sigatoka without affecting bunch weight or fruit quality (Carlier et al 2000; Calvo and Bolaños 2001). Plants are spaced to prevent overlapping of leaf canopies and to make the microclimate less favourable for disease development (PCARRD 1988). Plantations are sprayed from the air with fungicides at 8–21 day cycles (PCARRD 1988). The management of fungicide resistance is a significant issue (Slabaugh 1990; Marín et al 2003). Systemic fungicides such as strobilurons and propiconazole are usually applied in combination or alternation with broad-spectrum fungicides such as chlorothalonil or dithiocarbamates (Marín et al 2003). Petroleum-based oils may also be used (Vawdrey et al 2004).

Other *Mycosphaerella* diseases of bananas

Bananas are host to a number of pathogenic species of *Mycosphaerella*. Three species of *Mycosphaerella* cause Sigatoka-like symptoms. The yellow Sigatoka pathogen *M. musicola*, the black Sigatoka pathogen *M. fijiensis*, and the eumusae leaf spot pathogen *M. eumusae* have all evolved in the South-East Asian and Papua New Guinean region that is the centre of origin of edible bananas (Carlier et al 2000; Crous and Mourichon 2002; Hayden et al 2003a, 2003b). The banana speckle pathogen *M. musae* causes a serious disease in subtropical areas of Australia (Pont 1960b), but is relatively minor in other areas of the world (Jones 2000). All these species are restricted to the Musaceae. In the Americas there is a further species, *M. minima*, which is also restricted to bananas. *M. thailandica* occurs on *Acacia* and *Musa*, and *M. citri* occurs on *Citrus*, *Acacia* and *Musa* (Crous et al 2001).

Mycosphaerella fijiensis and *M. musicola* are morphologically very similar, especially their sexual stages (Meredith 1970; Mulder and Stover 1976). Restriction fragment length polymorphism (RFLP) techniques (Carlier et al 2000) and sequence analysis of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Johanson and Jeger 1993; Johanson et al 1994; Carlier et al 2000; Crous and Mourichon 2002) show the two pathogens as separate species that are members of different clades and phylogenetically distinct (Goodwin et al 2001; Crous et al 2001). *Mycosphaerella eumusae* is closely related to *M. musicola* (Crous and Mourichon 2002).

The black Sigatoka pathogen is well suited to the environmental conditions prevailing in wet tropical coastal regions and can replace the yellow Sigatoka pathogen (Stover and Dickson 1976). This occurred within two to three years in Honduras (Stover 1980) and in less than five years in Costa Rica (Gauhl 1994). Lesion expansion and ascospore production is greater for *M. fijiensis* and this has probably given it a competitive advantage over *M. musicola*. However, the situation is different at higher altitudes, as *M. musicola* seems more suited to cooler environments. Records showing that black Sigatoka is gradually becoming dominant at increasingly higher altitudes suggest that *M. fijiensis* may be slowly adapting to cooler temperatures (Carlier et al 2000).

In South-East Asia, *M. fijiensis* was recorded as present in Indonesia, western Malaysia and Thailand in the 1960s, but it has not become the dominant leaf spot pathogen (Carlier et al 2000). Other pathogens, such as *M. eumusae*, appear to be common and widespread throughout southern and South-East Asia (Crous and Mourichon 2002). In western Malaysia and Thailand, *M. eumusae* may be out-competing *M. fijiensis* (Carlier et al 2000).

Appendix 7. Freckle

Scientific name

Teleomorph

Guignardia musae Racib. 1909 [Dothideales: Mycosphaerellaceae]

Kirk et al (2001) place *Guignardia* in the Family Botryosphaereaceae.

Anamorph

Phyllosticta musarum (Cooke) van der Aa 1973

Synonyms

Sphaeropsis musarum Cooke 1880
Macrophoma musae (Cooke) Berl. and Voglino 1886
Phoma musae (Cooke) Sacc. 1918 non *Phoma musae* (P Joly) Boerema, Dorenb. and HA Kerst. 1965
Phoma musae Carpenter 1918
Phyllostictina musarum (Cooke) Petr. 1931

Andromorph

Leptodothiorella sp.

Common names

Freckle, *Phyllosticta* leaf spot, black spot.

Hosts

The principal hosts of freckle are *Musa* species, including a range of edible banana and plantain cultivars.

There have been reports of *G. musae* on *Ensete superbum* in India (Nag Raj 1993) and on *Heliconia* species in Venezuela (Madriz et al 1991). However, these observations were not supported by pathogenicity tests and are regarded as dubious.

In Papua New Guinea, disease symptoms have been observed on the wild banana species *Musa acuminata* subsp. *banksii* and *M. schizocarpa*. Freckle is particularly severe on *M. acuminata* subsp. *banksii* x *M. schizocarpa* hybrids (Jones 2000). Symptoms have also been observed on *M. textilis* in the Philippines (Ocfemia 1927) and on *M. paradisiaca* in Indonesia (van der Aa 1973). Symptoms have not been observed on *M. balbisiana* (Jones 2000). No information was found on the susceptibility of other species of *Musa* or *Musaceae.*

The field susceptibility of cultivated bananas and plantains appears to vary between geographical areas. In Australia (Cape York Peninsula and Torres Strait Islands), Papua New Guinea and the South Pacific islands (listed in Table 7.1), disease symptoms occur on Bluggoe (ABB), but not on Cavendish (AAA). In South-East Asia and the Philippines, disease symptoms occur on both Bluggoe and Cavendish (Jones 2000). In Hawaii and Taiwan, ABB genome clones such as Bluggoe are resistant, but while some AAA genome clones such as Cavendish are susceptible, others such as Gros Michel are resistant (Meredith 1968; Hwang et al 1984; Tsai et al 1993).

The susceptibility of Dwarf Cavendish (Chuang 1981) and Giant Cavendish (Meredith 1968) fruit has been confirmed in inoculation experiments using conidia obtained from pure culture and freckle lesions, respectively. No information was found concerning the deliberate inoculation of other apparent hosts.

Table 7.1 Recorded field reactions of *Musa* species and cultivars to freckle disease

Key: HR – Highly Resistant; R – Resistant; S – Susceptible; HS – Highly Susceptible

Species and cultivars	Eastern Australia and South Pacific [A]	North-western Australia, South-East Asia, including the Philippines [E]	Hawaii [B]	Taiwan [C]	India [D]
Wild species					
M. acuminata x M. schizocarpa	HS				
M. textilis		S			
M. acuminata				HR	
M. acuminata subsp. *banksii*	S				
M. balbisiana	R		R	R	
M. schizocarpa	S				
AA					
Inarnibal	R	R		R	
Jari Buaya	R	R			
Pisang Lilin	R	R		HR	
SH 3362				HS	
Sucrier (syn. Pisang Mas)		S (less)	R	S	
AB					
Mitli					R
Mysore Mitli					S (less)
AAA					
Basarai					S
Cocos			R	HR	
Dwarf Cavendish	R	S	S	HS	S
Giant Cavendish	R	S	S	HS	
Grand Naine				HS	
Gros Michel	R		R	HR	
Hanuman					S (less)
Iholena	R		R		
Karibale					R
Lakatan		S		HS	
Pisang Masak Hijau (syn. Jamaican Lacatan)			S		
Pisang Nangka		S (less)			
Poyo				HS	
Red and Green Dacca	R		R	R	R
Robusta			S	HS	S (less)
Sakkarebale					R
Tu8				HR	
Williams				HS	
Yangambi Km5				HR	
AAAB					
SH 3436 (Goldfinger)	S			HR	
AAB					
Bargibale					S (less)
Eslesno			R		
Father Leonore			R		
French Plantain		S			
Horn Plantain	S	S	S	S	
Huamoa			R		
Iho-u			R		
Maia Maoli			R		

Species and cultivars	Eastern Australia and South Pacific [A]	North-western Australia, South-East Asia, including the Philippines [E]	Hawaii [B]	Taiwan [C]	India [D]
Mysore	S	S		R	S (less)
Nanjanagudu Rasabale					S
Pisang Raja (syn. King, Rajapuri?)		S (less)	S	HS	S
Pome (syn. Lady Finger)		S	S	HS	
Popouli			R		
Silk (syn. Latundan)		S	S	S	
Sugundhi					R
Walhu			S		
ABB					
Blue Java (syn. Ice Cream)	S		R	R	
Bluggoe	HS	S	R		
Kluai Teparot		S			
Monthan			R		
Pelipita		S			
Pisang awak (syn. Ducasse)	S	S		HR	
Saba (possibly BBB)		S		HS	

[A] *Jones (2000); APPD (2008); Condé (2001)*
[B] *Meredith (1968)*
[C] *Hwang et al (1984); Tsai et al (1993)*
[D] *Thammaiah et al (2001)*
[E] *Lee (1922); Ocfemia (1927); Jones (2000)*

Plant parts affected

Guignardia musae causes spotting diseases on the leaves and fruit (Meredith 1968). This spotting may extend to the flower bracts, leaf midrib and bunch stalks under conditions of severe infection (Meredith 1968). Symptoms have not been described on the pseudostem, although there is no reason to suspect any resistance in this tissue.

Distribution

Freckle has been recorded in 27 countries through South–East Asia, Oceania and India (Table 7.2). The strain that affects Cavendish bananas has not been recorded in Central or South America, or Africa. A strain that infects Lady Finger and Bluggoe bananas occurs in Australia (Condé 2001) but the strain that infects Cavendish bananas does not currently occur on mainland Australia (Table 7.1).

Table 7.2 Distribution list for freckle

Country	References	Country	References
American Samoa	Dingley et al (1981)	New Caledonia	Johnston (1965)
Australia	Jones and Alcorn (1982)	New Zealand	Jones (2000)
Bangladesh	Jones (2000)	Niue	Dingley et al (1981)
Bhutan	Jones (2000)	Pakistan	Jones (2000)
Brunei	Jones (2000)	Papua New Guinea	Shaw (1963)
China	Zhou and Xie (1992)	Philippines	Lee (1922), Jones (2000)
Cook Islands	Wardlaw (1961)	Solomon Islands	Jones (2000)
Fiji	Dingley et al (1981)	Sri Lanka	Wardlaw (1961)
Hawaii	Campbell (1926)	Taiwan	Chuang (1981)
India	Carpenter (1918)	Thailand	Wardlaw (1961)
Indonesia	Wardlaw (1961)	Tonga	Dingley et al (1981)
Malaysia	Jones (2000)	USA	Wardlaw (1961)

Country	References	Country	References
Myanmar	Wardlaw (1961)	Vietnam	Jones and Daniells (1988)
Nepal	Jones (2000)	Western Samoa	Dingley et al (1981)

Records from Africa (Congo and Zambia) (Wardlaw 1961) and the Caribbean (Dominican Republic, Jamaica and St Lucia) were not included, as symptom expression on infected *Musa* plants is unusual and it is considered that the pathogen responsible has been misidentified (Jones 2000). A record from New Zealand (Jones 2000) was not included because there are no specimens in official collections to verify the record (SR Pennycook, Landcare Research, Auckland, pers comm 19 January 2008).

Philippines

In the Philippines, Lee (1922) noted that black spot disease (freckle) was widespread through the Sulu Archipelago and the island of Mindanao on cultivars of *Musa sapientum*. He concluded that as these regions were sparsely populated and bananas had not been imported from the western hemisphere, freckle disease was either indigenous or of very long standing.

Freckle has become established in Cavendish banana plantations on Mindanao Island and was observed in August 2001 by the TWG Chairs during a visit to a Cavendish banana plantation near South Cotabato (BA 2002c). It has also been observed on ripened Philippine bananas on sale in New Zealand (Allen, pers comm., 16 May 2006).

Australia

In Australia, APPD (2008) lists 66 records of *G. musae* on *Musa* spp., including 44 in eastern Australia and 22 in north-western Australia (Table 7.3). Forty-three records are on unnamed *Musa* spp., mainly non-commercial cultivars in remote areas of Torres Strait and Cape York Peninsula. There is one record on an unnamed *Musa* sp. on Christmas Island.

Of the 23 records on named cultivars, 11 are in eastern Australia and include the cultivars Bluggoe (ABB), Blue Java (ABB), Ducasse (ABB), Horn Plantain (AAB) and Goldfinger (AAAB). Of the remaining 12 records, two records are on Lady Finger (AAB) in the Northern Territory and 10 are on Cavendish (AAA) at Kalumbaru in the north-western part of Western Australia. The outbreak at Kalumbaru has since been eradicated by destroying all host plants at the original detection site and on neighbouring properties (Condé 2001). There are two records listed on Cavendish banana leaf from Kununurra, Western Australia, but repeated surveys since 1982 have not found the disease in that area (R Shivas, Plant Pathologist, Queensland Department of Primary Industries and Fisheries, pers comm. 18 February 2008). Freckle disease is considered not to be present in Western Australia.

Table 7.3 Records of *Guignardia musae* on *Musa* spp. in Australia

Source: APPD (2008)

Region	Records listed as *G. musae*	Host listed as unnamed *Musa* sp.	Host listed as named cultivar of Musa sp.	Comment
Eastern Australia				
○ Torres Strait islands	24	23	1	Bluggoe (1)
○ Cape York Peninsula	8	7	1	Bluggoe (1)
○ North Queensland	7	0	7	Blue Java (2); Bluggoe (2); Ducasse (2); Horn Plantain (1)
○ South-east Queensland	1	1	0	
○ New South Wales	4	2	2	Goldfinger (2)
North western Australia				
○ Christmas Island	1	1	0	

Region	Records listed as *G. musae*	Host listed as unnamed *Musa* sp.	Host listed as named cultivar of Musa sp.	Comment
o Northern Territory	6	4	2	Lady Finger (2)
o Western Australia	15	5	10	Cavendish (8 at Kalumbaru, 2 at Kununurra)
Total	**22**	**10**	**12**	

A survey undertaken in the Northern Territory recorded freckle from a number of locations (Condé 2001). It was detected on Lady Finger (AAB) and Bluggoe (ABB), but not on any Cavendish (AAA) bananas. Additional information from Queensland (S Perry, Plant Pathologist, Biosecurity Queensland, pers comm. 30 January 2008) lists freckle also on the cultivars Mysore (AAB), TU8 (AAAA) and the uncharacterised indigenous cultivars Warwar, Goly Goly Pot Pot, Simoi, and Tereg, in addition to the cultivars listed in Table 7.3. There is one record of freckle on Dwarf Cavendish (AAA) on Sabai Island in the Torres Strait.

Freckle is commonly seen on susceptible cultivars on Torres Strait islands, Cape York Peninsula and north Queensland, especially on cv. Bluggoe. Importantly, freckle has not been observed on any commercial Cavendish AAA or Lady Finger AAB bananas in eastern Australia and it is subject to official control should it occur on these cultivars.

Biology

The following review of the biology draws on literature published for *G. musae* and closely related fungi such as *G. bidwellii* on grapes and *G. citricarpa* on citrus. It also draws on literature published for other fungi that produce pycnidia.

The descriptions of *G. musae* are based on van der Aa (1973), Meredith (1968) and Chuang (1981). The description given by Punithalingam (1976) and Punithalingam and Holliday (1975) is erroneously based on a fast-growing saprophyte (Chuang 1981).

The teleomorph named *G. musae* is used for the freckle pathogen which may be found in four forms:

Mycelial thallus

Guignardia musae has a haploid mycelial thallus. The fungus is very slow growing, both on the host and in culture media (Chuang 1981).

The thallus occurs briefly on the surface of host tissues as a mycelial filament during the germination process. The cytoplasm of a germinating conidium (see *Anamorph* section below) transfers to the germ tube, which quickly forms a lobed appressorium at its tip. Most appressoria are formed in depressions between adjacent epidermal cells (Meredith 1968). After 18–30 hours, the appressorium becomes fully melanised, thick-walled and separated from the spore by a septum. Appressorium formation occurs at temperatures of 15–35 °C (Pu et al 2008).

Penetration of the host cuticle subsequently occurs via a fine infection peg from the appressorium (Meredith 1968; Pu et al 2008). *G. musae* enters the epidermal cells of the host directly and there is no evidence of infection via stomatal pores (Meredith 1968). Penetrated epidermal cells rapidly become reddish-brown in colour. Meredith (1968) noted that more than 60% of observed appressoria were associated with necrotic epidermal cells after 96 hours incubation. Infections occurred in association with single appressoria but symptom development was more rapid and extensive if multiple infections occurred simultaneously in close proximity.

Subsequent invasion occurs through intercellular spaces and directly between cells, but is restricted to the outer five layers of the mesophyll. Invaded cells become reddish-brown in colour within 96 hours of spore germination (Meredith 1968). A mycelial stroma is formed in the invaded host cells in which pycnidia develop over the following three weeks. By this time, some pycnidia have matured and are able to exude conidia.

Anamorph

The anamorph or asexual stage of *G. musae*, *Phylosticta musarum*, produces pycnidia and conidia.

The pycnidia are prominent under the raised host epidermis, mostly epiphyllous, sometimes amphigenous, single or clustered within diseased tissue, unilocular, 60–170 µm in diameter (mostly 135 µm) globose, pyriform or tympaniform, the upper region firmly grown together with the host epidermis and the subepidermal stroma, conspicuously papillate, with a 10–20 µm diameter pore. The stroma is presenchymatous, clyperous-like developed around the upper part of the pycnidia, subepidermal and composed of very dark hyphae. The pycnidial wall is hardly developed, only composed of some compressed brownish hyphae or brownish, thin walled cells, but 1–4 cells thick in the upper part, composed of flattened, dark brown cells, darkest around the pore and hyaline towards the conidiogenous end.

Mature pycnidia can be found in freckle lesions from about three weeks after infection. The numbers of pycnidia in lesions increase with increasing numbers of appressoria formed in the area. Meredith (1968) reported that a single pycnidium may be associated with fewer than five necrotic cells but, where there are multiple appressoria, there may be about 5 pycnidia per mm^2 of lesion surface. Small lesions typically had from 1–5 pycnidia per lesion, while the largest lesions had up to 70 pycnidia per lesion.

Conidia are formed on evanescent conidiogenous cells within the pycnidium, as is characteristic of the genus *Phyllosticta* (Kiely 1949; Van der Aa 1973). It is not known if *G. musae* pycnidia continue to produce conidia over an extended period of time but, in the related fungus *G. citricarpa,* fresh conidia are produced repeatedly from pycnidia subjected to alternating wet and dry cycles (Kiely 1949).

Conidia are one-celled, obovoidal, ellipsoidal or short cylindrical, pyriform when young with a truncated base, apically broadly rounded and conspicuously indented, 10–20 × 6–13 µm (Chuang 1981), surrounded by a 1–3 µm thick slime layer, containing a rather homogeneous mass of coarse, greenish guttules and usually one large bright vacuole; bearing an apical appendage up to 8–16 µm long, very distinct when present but often absent.

Conidia exude from mature pycnidia that have become wet and are either dispersed in water droplets immediately or after re-wetting of dried mucilage. In *G. bidwellii,* conidia settle from the water film and attach to the host surface over a period of 2–3 hours. After this time, they cannot be removed by washing (Spotts 1980). The attachment is thought to be mediated by Ca^{++} and to be a necessary precursor for successful germination and appressorium formation (Kuo and Hoch 1995).

Conidia produced naturally and in culture will germinate with a single germ tube on glass slides and host surfaces, starting in the presence of a water film after 2–3 hours and continuing over the next 24-96 hours (Meredith 1968; Chuang 1981). Pu et al (2008) observed that over 80% of conidia on banana leaves germinated after 36 hours incubation but only 56% of these successfully germinated and formed appressoria. They reported that nearly 99% of germinated spores produced appressoria after 120 hours incubation, although this may be an overestimate because their methods did not take account of spores that failed to attach or were subject to microbial lysis after prolonged incubation.

No information has been found on the numbers of freckle conidia that can be produced in a pycnidium. Meredith (1968) obtained fresh conidia for germination and pathogenicity experiments by thoroughly cleaning the surface of infected tissue and then incubating the cleaned tissue in a damp chamber for 48-72 hours. After this time, newly forming conidia exuded in mucilaginous masses from the pycnidia and could be suspended in water.

Spotts (1980) collected conidia from leaves infected with known numbers of *G. bidwellii* pycnidia during periods of continuous natural rain. It was found that conidia were released over a period of 4 hours from the onset of rain, commencing after 15 minutes and reaching a peak rate of discharge of 20 conidia per 15 minutes after 1.5 hours of continuous rain. Approximately 120 conidia were released per pycnidium over the 4 hour collection period (Figure 7.1). The pycnidia of *G. bidwellii* are of a similar size (56-196µm diameter) to those of *G. musae* (60-170 µm diameter).

Apparently dormant pycnidia can resume the production of conidia when re-wetted and this process can be repeated for a number of cycles until nutritional reserves are exhausted (Kiely 1949). The time between successive cycles of spore release is likely to involve several hours as it requires the production of fresh conidiogenous cells in the pycnidium (Kiely 1949). The rate at which this occurs is likely to be affected by temperature as it is an active process of development.

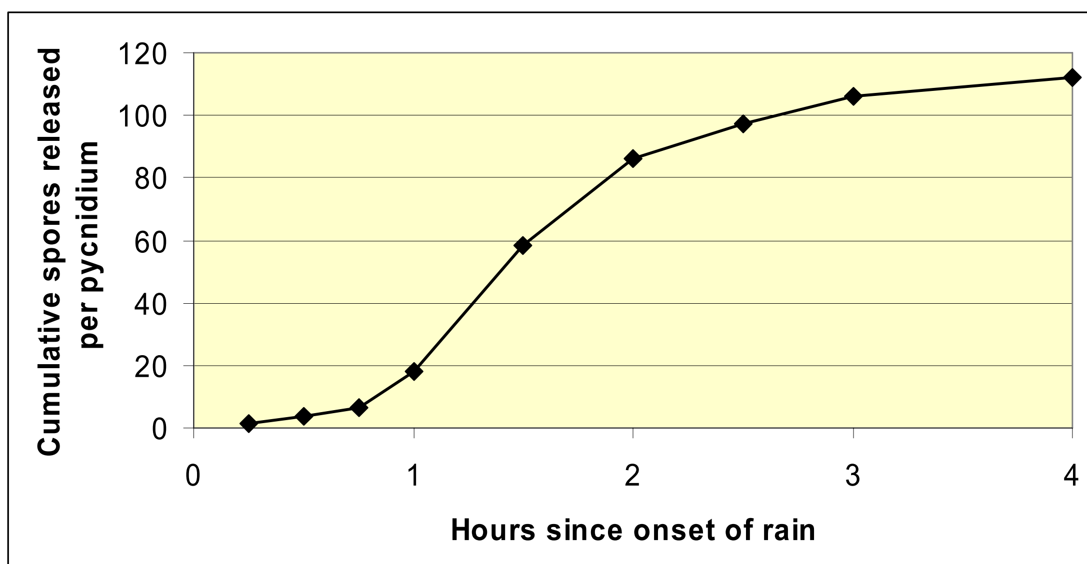


Figure 7.1 Release of conidia from *Guignardia bidwellii* pycnidia
Adapted from data in: Spotts (1980)

Andromorph

Spermogonia develop amongst pycnidia in infected tissues (Carpenter 1918). They are similar to pycnidia and possess a papilla with a pore approximately 20 µm wide. The spermogonial wall is only well-developed in the upper part. Spermatiogenous cells are cylindrical, slender, tapering towards the apex and 5–20 × 1–2 µm in size. Spermatia are one-celled, cylindrical or dumbbell-shaped, 6–10 × 0.5–2 µm in size and usually biguttulate.

Teleomorph

Guignardia musae, the sexual stage of the freckle pathogen, forms ascomata, asci and ascospores. The occurrence of saprophytic *Guignardia* spp. on *Musa* spp. can be mistaken for *G. musae* (Chuang 1981).

Ascomata are globose or somewhat depressed, 70–220 µm in diameter and distinctly papillate. The wall is 1–12 cells thick, composed of brownish, isodiametrical cells and sometimes flattened at maturity. Asci are 35–85 × 20–25 µm in size, cylindrical or clavate, usually with a short stalk, broadly rounded apically, bitunicate and contain 8 spores. Ascospores are 1–3 seriate, one-celled, ovoidal or oblong-ovoidal and 17–22 × 8–10 µm in size.

Jones (2000) considered that the role played by the sexual stage of *G. musae* in the epidemiology of freckle disease was unclear. The teleomorph was first described in 1909 on leaves of *M. paradisiaca* collected near Bangor, Indonesia (van der Aa 1973). This area includes tropical lowlands and temperate highlands but no details were provided on where the specimens were collected or the disease prevalence. The teleomorph has been found elsewhere only in Taiwan. Dr Chao advises that Prof Chuang in 1982 reported that ascospores could be observed in diseased leaves sampled from the field in the dry winter season (CP Chao, Plant Pathologist, Taiwan Banana Research Institute, pers comm 14 February 2006). These ascospores were released from infected leaf tissue about 15 minutes after the tissue was moistened and 50% of the ascospores present in the sample were released within two hours. Almost no ascospores could be found in infected foliage collected in the hot and wet season

in Taiwan. The ascospores released from diseased tissue were shown to be pathogenic to Cavendish bananas.

Tsai et al (1993) noted that ascospores appeared to initiate infections in newly planted Cavendish bananas derived from tissue culture when rain occurred towards the end of the cool dry season in Taiwan. It was considered that the presence of the teleomorph in diseased tissue might contribute to the overwintering of the fungus, as is the case with *G. citricarpa* (Kiely 1949) and *G. bidwellii* (Ferrin and Ramsdell 1977). Kiely (1949) noted that the release of ascospores enhances the opportunities for inoculum produced at ground level to infect the uppermost leaves, when compared with splash-dispersed, conidial inoculum.

In Hawaii, Carpenter (1918) noted that spermogonia occurred in association with pycnidia, although at a lower frequency than pycnidia, but could not find any ascomata. Ocfemia (1927) and Meredith (1968) did not comment on the occurrence of ascomata in diseased tissue taken from the field in the Philippines and Hawaii, respectively, noting only the abundance of pycnidia in diseased tissue.

It has been noted that the tropical lowland and highland banana-growing areas of the Philippines do not have a cool dry season like that in Taiwan, and therefore may not have conditions conducive to the development of the teleomorph *G. musae.* It has also been noted that the conditions in subtropical banana growing areas of Australia are very similar to those in Taiwan and may be conducive to the development of the teleomorph. The situation with respect to North Queensland is less clear because the tropical wet season is followed by a cool, relatively dry season.

Sexuality

No information has been found on the sexuality of *G. musae*, although it is expected to be heterothallic like other Loculoascomycetes. Kiely (1949) drew attention to the presence of spermogonia in *G. citricarpa* and proposed that the spermatia were involved in the production of ascomata. The existence of mating types has not been reported for *G. musae*.

Detection and identification

Leaf symptoms

Fungal infection and disease symptom expression can occur on both young and old leaf tissues. Two kinds of leaf spot symptoms have been described (Meredith 1968).

One consists of very small (less than 1 mm) dark brown to black spots, mainly on the upper surface of the leaves, which give them a sooty appearance. Numerous pycnidia develop and protrude slightly through the cuticle, which gives a rough feel to the leaf surface. Spots can cluster in streaks that may run diagonally or horizontally across the leaf. In other cases, these streaks run along the veins from the midrib to the edge of the leaf. Yellowing of the leaf occurs where freckling is severe.

The second kind of spotting is characterised by relatively large (up to 4 mm in diameter) individual dark brown to black spots. The spots may have grey centres and can aggregate to form large blackened areas or streaks with yellowish green haloes. Pycnidia are very prominent and raise the epidermis to give the diseased leaves a rough feel. Severely affected leaves turn yellow, wither and die prematurely (Meredith 1968). When the transition leaf collapses as a result of infection, it provides an abundant source of inoculum for lower leaves and the developing fruit.

Chuang (1984) reported that the incubation period of freckle disease could be as long as 60 days. This observation was made after examining marked banana leaves in the field. The age of each leaf when symptoms of freckle first appeared was noted. Symptoms first appeared on 60 day-old leaves in December 1979, June 1980 and December 1980. Extended periods of incubation were also observed in November 1981 and February 1982.

In all cases, the extended period of incubation coincided with an extended period of dry weather. It is evident that the extended period of incubation was related to the absence of water films on the leaf surfaces. As it is unclear when infection first occurred in these experiments, there is some doubt about

whether the incubation period was as great as indicated by the observations. When rainy conditions occurred after leaves emerged, the incubation period for freckle disease was 20–30 days, which is consistent with the results of inoculation experiments (Meredith 1968; Chuang 1981).

Fruit symptoms

Disease symptoms are rarely seen on banana fruit in the absence of leaf symptoms. They are particularly severe when the fruit is in contact with or adjacent to diseased leaves and often more severe on the large hands at the top of the bunch than those at the bottom (Meredith 1968).

Fruit is susceptible to infection by *G. musae* from the time of bunch emergence until maturity (Meredith 1968; Chuang 1984). On very young fruit, individual spots first appear as minute red brown flecks, surrounded by a halo of up to 2 mm of dark green water-soaked tissue. Meredith (1968) reported that during rainy periods in Hawaii, spots may occur as a small number of widely scattered spots or as dense aggregates in streaks or circles. In the field, symptoms appeared on immature fruit 2–4 weeks after the bunch emerged from the pseudostem. Disease severity increased as the fruit matured, as a result of secondary infections.

In experiments involving inoculum obtained from diseased leaves, Meredith (1968) reported that when Dwarf Cavendish fruits 10–20 days old were inoculated with conidia of *G. musae*, symptoms appeared within four days. Spots enlarged over the following 10–20 days and mature pycnidia developed 21 days after inoculation.

Chuang (1984) reported that the incubation period of freckle on Giant Cavendish fruit decreased with increasing age of the fruit at the time of inoculation. When young fruit (10 days old) were inoculated and incubated under ambient conditions in Taiwan, symptoms appeared after about 40 days from the time of inoculation. When mature fruit (120 days old) were inoculated and incubated under similar conditions, symptoms appeared after 22–29 days from the time of inoculation, depending on whether natural or artificial inoculum was used, respectively (see Figure 7.2).

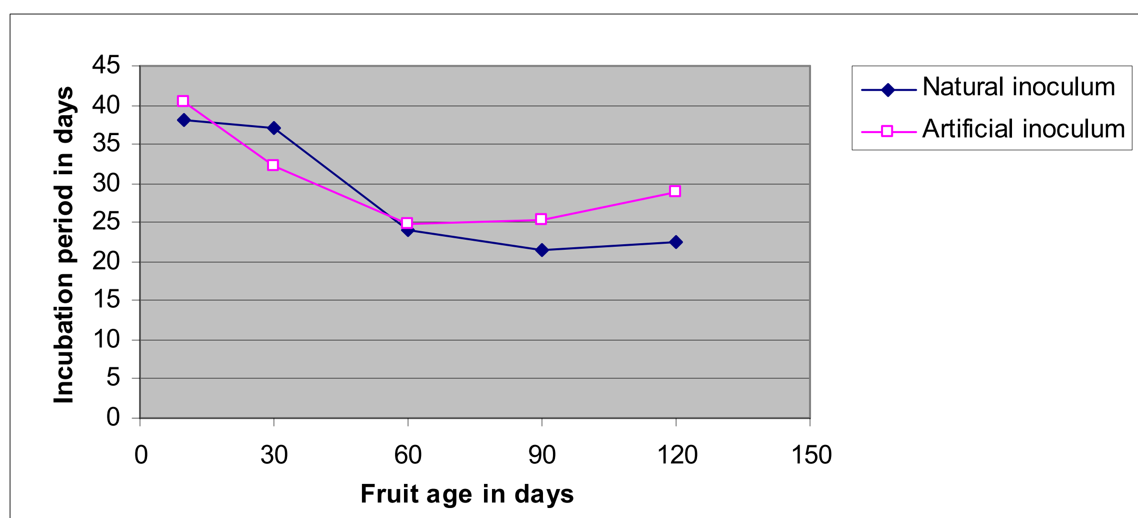


Figure 7.2 Effect of age at inoculation on the incubation period of freckle disease
Source: from Chuang (1984)

In other experiments, six hands of fruit at sequential positions on the bunch were inoculated with conidia produced naturally or artificially in culture. Hand 1 was the large hand at the top of the bunch and hand 6 was at the lowest end of the bunch. Fruit were inoculated when the bunch was 30 days old and then observed for the development of disease symptoms. The incubation period varied from 14–23 days (Figure 7.3), but the differences in incubation period between hand positions were not statistically significant (P=0.05). Chuang (1984) did not comment on the presence of pycnidia in the freckle lesions produced in these experiments.

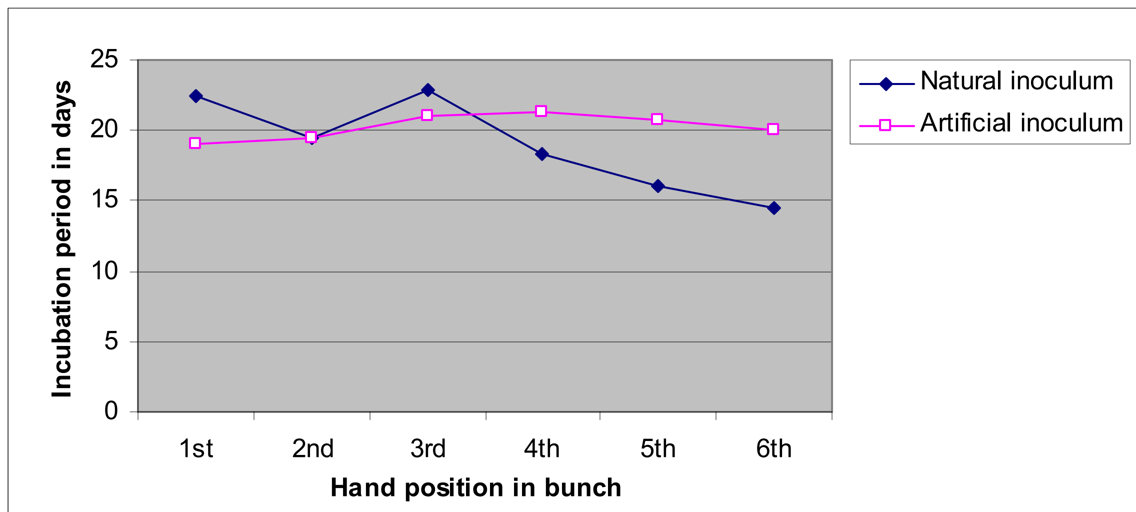


Figure 7.3 Effect of hand position in the bunch on the incubation period of freckle

Fruit was inoculated with natural and artificially produced conidia of G. musae when the bunch was 30 days old. Source: from Chuang (1984).

As the bunch matures, secondary infections result in large areas of the fruit surface and peduncle becoming black from the dense aggregation of spots. These spots are particularly noticeable at the time of harvest (Meredith 1968). During ripening, the individual spots are surrounded by a halo of green tissue up to 3 mm in diameter (Meredith 1968). On the outer face of the fingers of a cluster, freckles are most abundant towards the blossom-end whereas, on the inner face, freckles are concentrated towards the stalk end (Meredith 1968). Streaks may run the entire length of the banana.

The number of spots produced per square centimetre has been used to measure disease severity in Taiwan (Chuang 1984). For example, in October 1981 approximately 40 freckle lesions per cm^2 were found on fruit surfaces following extended wet weather in the previous July–September period. From February to June 1982, there were only 2–8 freckle lesions per cm^2. The reduced disease severity followed a period of abnormally dry weather from October 1981 to April 1982.

Strains of *G. musae*

Differences in its relative ability to attack various banana lines, in particular the AAA Cavendish subgroup and the ABB Bluggoe subgroup, suggest that more than one type or strain of *G. musae* may exist (Table 7.4). Jones (2000) suggested there are two strains of *G. musae*, one that attacks Bluggoe but not Cavendish and occurs in Australia and in the South Pacific, and another that in Hawaii attacks Cavendish but not Bluggoe. However, in South Asia and South-East Asia, there are strains that attack both Cavendish and Bluggoe bananas (Table 7.1). Research is needed to determine if these different pathotypes can be distinguished using morphological or molecular methods. Further research is also needed on the pathogen causing banana freckle in Africa and the Caribbean to determine whether it matches the description of *G. musae* given by van der Aa (1973).

There is currently no information to establish whether there are multiple races of *G. musae,* or if there are several species of fungi causing freckle on species of *Musa, Heliconia* and *Ensete*. A preliminary study of the DNA obtained from a collection of *G. musae* from bananas and plantains in the geographic areas defined in Table 7.4, indicates the presence of three genotypes in *G. musae* (van Brunschot and Henderson 2005). Clade 1 consists of isolates from Queensland, Torres Strait, Papua New Guinea, Indonesia, Northern Territory and Western Australia. Clade 2 is represented by samples from Indonesia, East Timor, Malaysia, Vietnam, Sri Lanka, Northern Territory and Western Australia. Clade 3 has been found only in Sri Lanka. The evidence suggests that Clade 2 has not yet reached Cavendish banana plantations in eastern Australia. No correlation could be made between host cultivar and pathogen genotype on the available evidence, as all three genotypes of *G. musae* were found on a

range of host banana cultivars. However, van Brunshot and Henderson (2005) express an opinion that the evidence challenges the suggestion by Jones (2000) of a 'Cavendish competent' strain.

Table 7.4 A summary of pathotype and genetic variation in *G. musae* between regions

***Key**: R – field resistant; S – field susceptible; P – detected in this region; nd – not detected in this region; ? – not tested from this country or region*

Pathotype or clade	Eastern Australia and South Pacific	North-western Australia, South-East Asia	Philippines	Hawaii	Taiwan	India	Sri Lanka
Field disease[1]							
Cavendish	R	S	S	S	S	S	S
Bluggoe	S	S	S	R	?	?	?
Molecular studies[2]							
Clade 1	P	P	?	?	?	?	nd
Clade 2	nd	P	?	?	?	?	P
Clade 3	nd	nd	?	?	?	?	P

[1] Jones 2000; Condé 2001; Meredith 1968; Hwang et al 1984; Tsai et al 1993; Thammaiah et al 2001
[2] van Brunschot and Henderson 2005

Other species of *Guignardia* associated with *Musa*

A number of other species of *Guignardia* and *Phyllosticta* infect species and cultivars of *Musa*. Some of these are weakly pathogenic saprotrophs that colonise *G. musae* lesions and quickly become the dominant species. For example, the fungus that Punithalingam and Holliday (1975) described as *G. musae* has been shown to be a saprotroph of diseased banana tissue (Chuang 1981).

The fungal species associated with freckle symptoms on *Musa* in West Africa and the Caribbean may be different from *G. musae*. *Guignardia sydowiana* was described from senescent leaves of *Musa* species from the Congo (Photita et al 2002). *Guignardia cocoicola* causes asymptomatic endophytic infections of leaves of wild *M. acuminata* in Thailand (Photita et al 2001). *Guignardia mangiferae* has been found to be associated with leaf spots and endophytic infections of *Musa* species in New South Wales (Baayen et al 2002). In addition, a fungus resembling *G. citricarpa* has been recorded on banana fruit (host listed as *M. acuminata*) grown at Ormiston, south-east Queensland (APPD 2008).

One of the more prevalent endophytic fungi found in leaves of *Musa acuminata* growing in Hong Kong was *Phyllosticta musicola* Sawada, originally described from Taiwan (Brown et al 1998). In the same study *P. musicola* was not isolated from leaves of commercial bananas or *M. acuminata* subsp. *banksii* growing in Queensland. Two other species of *Phyllosticta* have been described from *Musa,* but little is known about their biology or pathogenicity. They are *P. musae* from Hawaii and *P. musae-sapientium* from the Dominican Republic.

Epidemiology

Effects of temperature

Figure 2.1 and Figure 2.2 (Appendix 2, Part C of this report) show the average monthly temperatures for two Philippines areas and eight Australian cities in both grower and non-grower areas. The temperatures relevant to the importation of banana fruit are the environmental temperatures in the Philippines and Australia and also those present during transport (at 13–14 °C for 7–10 days) and ripening (at 14.5–21°C).

Meredith (1968) conducted experiments at 24 °C, while Chuang (1984) made reference to unpublished work concluding that the most appropriate temperature for growth of *G. musae* was 22 °C. Chuang (1984) also noted that appressoria were formed at temperatures of 17–22 °C

Pu et al (2008) found that the optimum temperature for spore germination on banana leaves was 25 ºC but not much different over the range of 20-30 ºC. However, at 15 ºC and 35 ºC the rates of

development were about half of those at the optimum temperature. Appressorium formation lagged about 6 hours behind germination, consistent with observations by Meredith (1968). In two experiments (Figure 7.4), appressoria were first observed after 6 hours incubation at favourable temperatures and their formation reached a peak of about 80% after 48 hours. The rate of appressorium formation at 15 ºC was about half of that at favourable temperatures. Pu et al (2008) did not test growth at temperatures of less than 15 ºC although the results in Figure 7.4 indicate that growth may occur at lower temperatures.

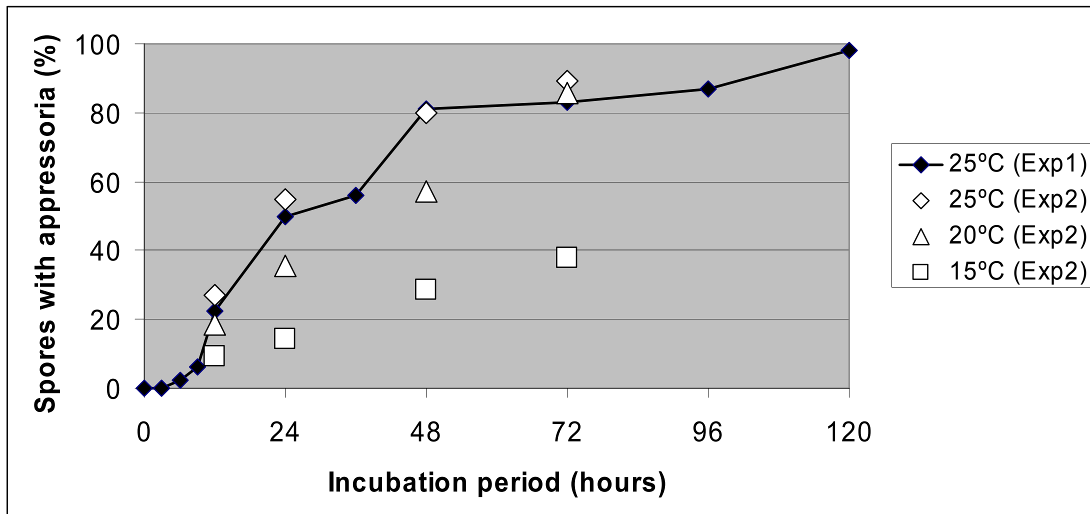


Figure 7.4 Time course of appressorium formation by *G. musae*

Source: based on two experiments reported by Pu et al (2008)

Studies on black rot of grapes (*G. bidwellii*) could be relevant due to the biological similarity of the epidemiology of *G. bidwellii* and *G. musae*. In growth chamber experiments, Spotts (1977) reported that the incubation period for black rot on grape leaves was 13.5 days at 15 °C compared with 7.5 days at 26.5 °C. Pycnidia appeared 19 days after inoculation when incubated at 15 °C compared with 12 days at 26.5 °C. Similar results were obtained in the vineyard. Spotts (1977) also reported that for light infections the minimum length of time that the leaf needed to be wet varied from 6 hours at 26.5 °C to 24 hours at 10 °C (Figure 7.5).

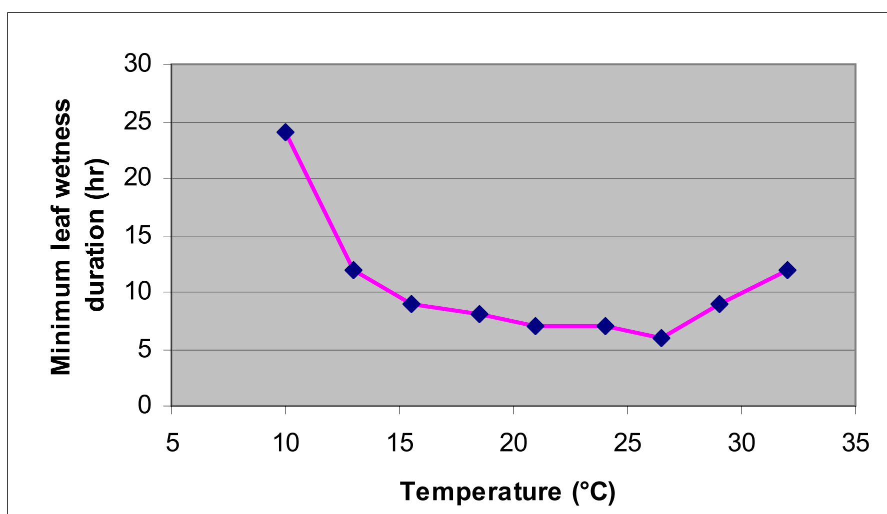


Figure 7.5 Period of wetness required for light infection of grape leaves by *G. bidwellii*

Source: based on data from Spotts (1977)

Effects of moisture

Free water is required for spore production, dissemination, germination and appressorium formation. This moisture can be provided by both rain and dew (Meredith 1968).

The production of conidia requires the pycnidia to be thoroughly wet. Meredith (1968) incubated pycnidia at 24 ºC in damp chambers for 48–72 hours to obtain fresh conidia for experimental purposes. Spotts (1980) found that the maximum rate of release of *G. bidwellii* conidia occurred approximately 1.5 hours after the pycnidia first became wet, although some conidia were released within 15 minutes of the onset of rain (Figure 7.1)

Conidia of *G. musae* suspend readily in water, even after the mucilage has dried, and are subsequently dispersed in run-off water or rain-splash. Conidia were found in almost every droplet reflected from an infected host surface after impaction of a 65 µl water drop, especially when the plant surface was already wet. The concentration of conidia in these droplets was in the order of 10^5–10^6 per ml.

Meredith (1968) found that fresh conidia were deposited on fruit surfaces almost every day by rainwater and dew that had dispersed spores from freckle lesions. Conidia were always present in dew droplets falling from infected leaves between 5 and 7 am. More than 80% of these spores germinated in a water film after 12 h incubation at 24 ºC.

Both Meredith (1968) and Chuang (1984) commented on the close association between freckle disease severity and rainfall. Chuang (1984) provided extensive field data that demonstrated this association. In Taiwan, Chuang (1984) considered that a continuous 48-hour period of wetness was required for successful appressorium formation and subsequent infection. Pu et al (2008) provided confirmation of this prediction (Figure 7.4).

It is unusual for periods of rainfall or surface wetness to persist for 48–hours in Australia. The Australian Bureau of Meteorology provides data on various amounts of daily rainfall per year (see Figure 2.4 of Appendix 2). In populated areas where most banana waste is generated, there is an average of 73–106 days per year with more than 1 mm of rain per day (Allen 2008). The likelihood of two successive days of rain would be less than that for a single day.

Allen et al (1992) collected surface wetness data for a number of sites in banana growing areas of Australia and Allen (2008) re-analysed the data from Modanville and South Johnstone. From January to December 1991 at Modanville, New South Wales, more than 18 hours of surface wetness occurred on only six days of 354 days counted. There were no periods when wetness continued for the full 24 hours. About 80% of hourly periods were dry and surface wetness persisted for up to 6 hours after wetting. A similar pattern emerged at a wetter site at South Johnstone, North Queensland. From July 1991 to June 1992, there were 20 days of 330 days counted with more than 18 hours of surface wetness per day but no days were wet for a full 24 hours. About 30% of hourly periods were dry but surface wetness persisted for up to 7 hours after wetting. There was a correlation between monthly averages of rainfall and surface wetness, indicating that 1 mm of rainfall led to 1–2 hours of wetness in the day and night. There was an additional 1-2 hours of dew at night even without rainfall. It is noted that the years 1991 and 1992 were drier than average so the number of days of rainfall and wetness may be lower than normal, but the correlation between surface wetness and rainfall should still hold.

Allen (2008) examined daily rainfall and surface wetness data from Alstonville, New South Wales, for the period from 1 October 1991 to 13 May 2006. It was found that 67% of days had no rain and 53% of days had no surface wetness. Of the days when surface wetness was recorded, only 5% were wet for more than 12 hours and 0.3% for a full 24 hours. Wetness periods of 1 hour per day were associated with rainfall of more than 1 mm per day, while wetness periods of more than 18 hours per day were associated with at least 5mm of rain per day on average.

Dispersal

Meredith (1968) found that conidia dispersed in water mainly to the area within a few millimetres of the pycnidium they had emerged from. Here, they germinated and formed appressoria. He also

considered that run-off water dispersed conidia primarily to leaves and fruit beneath diseased leaves. Meredith (1968) did not define the distance of rain-splash dispersal but later (Meredith 1973) reviewed literature on the dispersal of fungal spores generally. Evidence for splash dispersal over a distance of up to two metres is summarised under black Sigatoka (Appendix 6 of Part C).

There is no evidence that conidia can be induced to become airborne. Meredith (1968) and Chuang (1981) noted the existence of mucilage associated with conidial tendrils emerging from pycnidia and how this mucilage dried to bind the conidia to the host surface. While this mucilage dissolved in the presence of water, there was no splash-dispersal until the mucilage became thoroughly wet.

The movement of infected fruit with mature lesions (with pycnidia) or immature lesions (pre-symptomatic and without pycnidia) may be an effective means for spreading the organism. Indeed, Lee (1922) speculated on the possibility of freckle disease being spread from the Philippines to Hawaii on a few infected fruits carried by sugarcane labourers. Movement on infected leaves attached to vegetative planting material or used for padding harvested fruit may be similarly effective in spreading the organism. Evidence for the occurrence of leaf and floral contaminants of Philippine fruit marketed in New Zealand is summarised under black Sigatoka (Appendix 6 of Part C).

There is no evidence that ascospores are involved in the dispersal of freckle disease in the Philippines or Hawaii. However, ascospores are involved in the dispersal of *G. musae* at the end of the cool, dry season in Taiwan (Tsai et al 1993; CP Chao, Pathologist, Taiwan Banana Research Institute, Taiwan, pers comm 14 February 2006). If ascospores were involved, it is expected that they would be released from asci and dispersed in the air in a manner similar to *M. fijiensis* (see Appendix 6 of Part C). Freckle lesions would develop on the tallest leaves in the plantation instead of only those leaves beneath infected leaves.

No information has been found on other potential vectors of *G. musae*, although the possibility of insect transmission has been raised by ABGC (2007). The roles of insects in dispersal of spores are summarised under black Sigatoka (Appendix 6 of Part C).

Survival

No information has been found on the survival of *G. musae* in banana waste material. Circumstantial evidence suggests that the epidemiology of freckle disease in tropical and subtropical banana plantations is closely related to the conidial inoculum produced in diseased leaves attached to living plants (Meredith 1968; Chuang 1984). However, pycnidia, appressoria and the mycelial thallus of *G. musae* are strongly melanised (Meredith 1968) and would appear to have the potential to survive for considerable periods in decayed plant material.

The related fungus, *G. citricarpa*, survives in citrus leaf litter for as long as the host tissue remains intact (Kiely, 1949). Both pycnidia and ascomata develop in infected leaf litter but the extent of development is restricted by competing organisms such as *Colletotrichum gloeosporiodes* (Kiely 1949) and the common endophyte, *G. mangiferae* (Baayen et al 2002). Unlike *G. citricarpa*, there is no evidence of an extended endophytic existence in *G. musae*.

Conidia have been found to germinate in water run-off from infected banana leaves (Meredith 1968). Spores freshly collected in this way had a germinability of more than 80%. However, the ability of *G. musae* conidia to survive drying after being disseminated in water has not been established. Meredith (1968) found that no infection occurred if host surfaces were dried within 12 hours of inoculation. Conidia of *G. bidwellii* do not survive post-inoculation drying for more than two hours (Spotts 1977), whereas conidia of *G. citricarpa* survive for at least four days when dried onto glass slides (Kiely 1949). It would appear that conidia survive rapid drying before germination commences but are vulnerable to drying once germination has commenced.

There is evidence that *G. musae* can overwinter as ascomata in leaf litter and release ascospores when this tissue becomes wet (CP Chao, Pathologist, Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan, pers comm 14 February 2006). It has the potential to produce large numbers of conidia from pycnidia embedded in decayed host plant material, but the survival time for pycnidia has not been

investigated. Nor has its potential to exist as a saprophyte on decayed organic matter been demonstrated.

Sporulation and infection events

There are no published criteria for defining sporulation or infection events for freckle similar to that used by Peterson et al (2005) for black Sigatoka. For freckle, the conditions for sporulation and infection are expected to differ. Infection events require that suitable conditions for sporulation occur immediately beforehand.

Sporulation events

The following information is considered relevant to defining appropriate criteria for a sporulation event:

- Meredith (1968) and Chuang (1984) found that conidia exude from mature pycnidia that have been thoroughly wet, sometimes within 15 minutes of wetting but more extensively after 24 hours incubation in moist conditions at optimum temperature. Meredith (1968) also found that conidia exude from mature pycnidia into water associated with dew and that these conidia germinated readily.
- Spotts (1980) found that pycnidia of *G. bidwellii* released conidia over a period of 4 hr from the onset of rain, commencing after 15 minutes and reaching a peak of 20 conidia per 15 minutes after 1.5 hours of continuous rain. Approximately 120 conidia were released per pycnidium over the 4 hour collection period (Figure 7.1).
- Allen (2008) found that several hours of surface wetness was associated with more than 5 mm of rain per day on average.
- Kiely (1949) found that conidia of *G. citricarpa* survived on glass surfaces for at least four days if dried rapidly after release from a pycnidium. However, other workers (Meredith 1968; Spotts 1977) have found that conidia do not survive for more than a few hours if dried after spore germination commences.
- The production of conidia is an active process involving the development of evanescent conidiogenous cells (Kiely 1949), which is dependent partly on temperature. The effects of temperature on conidial production are expected to be similar to the effects on germination and appressorium development. The rate at which conidia are produced at 10-20 ºC is expected to be half of that at 20-30 ºC (see *Effects of temperature* above).
- Rain is required to splash conidia from surface waste to susceptible host tissue (Kiely 1949; Meredith 1968; Huber et al 2006).

The above observations suggest the following criteria for a sporulation event:

- One day with >5 mm rainfall and an overnight minimum temperature of >20 ºC; or
- Two consecutive days with >5 mm rainfall per day and an overnight minimum temperature of >10 ºC.

These criteria were applied to metereological data from South Johnstone (representing exposure groups in north Queensland), Alstonville (representing exposure groups in south-eastern Queensland and north-eastern New South Wales), Sydney (representing exposure groups in the northern parts of other (non-banana growing) areas of Australia), and Melbourne (representing the southern parts of other areas of Australia). The data were recorded over 11 years from 1996 to 2006, inclusive. Table 7.5 shows that there were 107.1 sporulation events per year at South Johnstone on average (ranging from 80 to 145), 35.4 at Alstonville (range 20–61), 18 at Sydney (range 10–27), and 1.8 at Melbourne (range 0–3).

Table 7.5 Number of days that start a potential sporulation event.

Year	Melbourne	Sydney	Alstonville	South Johnstone
1996	3	19	48	100
1997	1	10	41	90
1998	3	24	23	114
1999	3	22	61	111
2000	3	13	22	145
2001	3	24	33	101
2002	0	17	27	84
2003	2	27	39	80
2004	1	14	31	116
2005	1	17	20	96
2006	0	11	44	141
minimum	**0**	**10**	**20**	**80**
mean	**1.8**	**18.0**	**35.4**	**107.1**
maximum	**3**	**27**	**61**	**145**

Infection events

The following information is considered relevant to defining appropriate criteria for an infection event:

- Kiely (1949) found that conidia of *G. citricarpa* could withstand desiccation for at least four days if dried rapidly after release from the pycnidium and before germination commences.
- Meredith (1968) found that no infection occurred if the leaf surface dried out within 12 hours of inoculation. Much more infection occurred if the leaf surface was kept wet for 48 hours or more. The incubation temperature was reported as 24ºC.
- Pu et al (2008) found that the optimum temperature for spore germination and appressorium formation on banana leaves was 25 ºC but not much different over the range of 20–30 ºC. However, at 15 ºC and 35 ºC the rates of development were about half of those at the optimum temperature.
- Spotts (1977) found that the minimum period of wetness required for infection of grape leaves by *G. bidwellii* increased from about 6 hours at favourable temperatures to 24 hours at 10ºC.
- Allen (2008) found that there was a correlation between the amount of rainfall and the duration of surface wetness. Wetness periods of more than 18 hours per day were associated with more than 5 mm of rain per day on average. Given that there needs to be a period of at least 12 hours wetness per day for successful infection (Meredith 1968), it is expected that a period of 5 mm of rain would constitute a day with moisture adequate for growth and survival of *G. musae* on a host surface.

The above observations suggest the following criteria for a freckle infection event -

- a sporulation event followed by two consecutive days with >5mm rainfall per day and an overnight minimum temperature of >20ºC; or
- a sporulation event followed by three consecutive days with >5mm rainfall per day and an overnight minimum temperature of >14ºC; or
- a sporulation event followed by four consecutive days with >5mm rainfall per day and an overnight minimum temperature of >10ºC.

These criteria were applied to the meteorological data used for calculating sporulation events (Table 7.5) to also calculate the number of infection events. An infection event in this case is conditional on there being a preceding sporulation event. Table 7.6 shows that there were 48.1 conditional infection

events per year at South Johnstone on average (ranging from 27 to 79), 3.6 at Alstonville (range 1–8) and 1.1 at Sydney (range 0–4). There were no such days for Melbourne during the period.

Table 7.6 Number of days per year that start a potential sporulation event followed by a potential infection event.

Year	Melbourne	Sydney	Alstonville	South Johnstone
1996	0	2	3	49
1997	0	0	3	33
1998	0	0	1	42
1999	0	2	8	44
2000	0	1	1	77
2001	0	1	6	41
2002	0	0	3	37
2003	0	4	4	27
2004	0	0	4	59
2005	0	1	1	41
2006	0	1	6	79
minimum	**0**	**0**	**1**	**27**
mean	**0.0**	**1.1**	**3.6**	**48.1**
maximum	**0**	**4**	**8**	**79**

Disease severity in the Philippines

Data provided by Philippine authorities (BPI 2002b) indicated that freckle disease is found in some Cavendish plantations with the implication that it is not found in all Cavendish plantations. The disease was observed on leaves in one Cavendish plantation by the TWG Chairs during a visit to Cotobato in August 2001 but the disease was not observed on Cavendish fruit in any of the plantations visited (Biosecurity Australia 2002c).

Survey data provided by BPI (2002b) indicated that freckle is found on Cavendish banana leaves all year round. The severity of disease varied from 8.6 in July 1998 (most severe) to 12.3 in November 2000, as measured by the youngest leaf spotted on unbunched plants (Figure 7.6). Similar data on the occurrence of freckle disease on fruit have not been provided. It is evident that if 8-12 of the youngest leaves remain free of disease on non-bunched plants, then the transition leaf and other leaves on bunched plants will remain free of disease for several weeks after bunch emergence. This is expected to minimise opportunities for inoculum to spread to fruit.

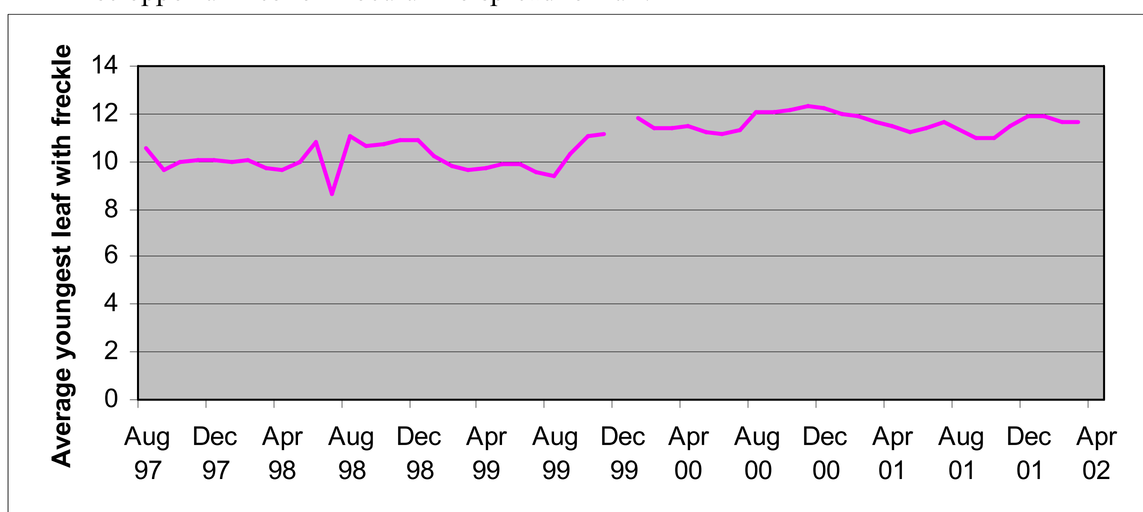


Figure 7.6 Average youngest leaf with freckle disease symptoms

Based on data provided by Philippines authorities (BPI 2002b) for unbunched plants in export Cavendish banana plantations on Mindanao Island in the period from August 1997 to March 2002.

In spite of the above information, mild symptoms of freckle disease have been observed on ripened Philippine bananas on sale in New Zealand (Allen, pers comm., 16 May 2006). The disease was identified by the presence of lesions with raised, black centres that are indicative of the presence of pycnidia (Meredith 1968). Two of four commercial lots were found to have symptoms, which involved from 1 to 23 individual lesions per finger scattered randomly over the fruit surface. The ripened fruit examined in this instance would have been harvested at least three weeks previously (refer to Part B, Section 7.2.5, *Packing and transport to Australia*). Given the controlled temperatures of 13-14 ºC for 10-14 days during shipping, it is expected that the symptoms observed in New Zealand would have resulted from infections that occurred before harvest. The occurrence of brown flecks, that would indicate recent post-harvest infections, was not reported.

Pest impacts

Economic impact

The disease does not affect the eating qualities of the fruit (Meredith 1968). However, the blackening of the fruit surface detracts from the appearance of the fruit and reduces its acceptance by consumers.

The impact of freckle disease on fruit production has been reported as being relatively minor in comparison to that of the leaf spot diseases caused by *M. musicola* and *M. fijiensis* (Meredith 1968; Stover 1972; Carlier et al 2000). This was related in part to the disease being most prevalent on the old leaves of the plant and partly to the effectiveness of fungicides otherwise used for the control of *Mycosphaerella* leaf spots. However, Tsai et al (1993) reported that freckle disease had gradually assumed greater importance in banana growing areas of Taiwan over the previous decade. In many areas, it replaced black Sigatoka (*M. fijiensis*) as the dominant leaf pathogen.

Disease control

Philippine authorities (BPI 2002b) have reported that freckle disease is controlled by the same fungicide and sanitation measures used against black Sigatoka disease. The fungicides are triazoles, strobilurin, tridemorph, mancozeb and chlorothalonil. The spray schedule is every one to three weeks, depending on weather conditions and disease status.

Guignardia musae pycnidia appear to be tolerant of surface disinfectant chemicals when embedded within diseased leaf tissue. Chuang (1981) treated leaf freckle samples with a 0.5% solution of sodium hypochlorite for two minutes to sterilise their surfaces before isolating *G. musae.* However, conidia of *G. musae* suspended in water are killed by low concentrations of chlorine under laboratory conditions (BPI 2002b). It is noted that the fungicide myclobutanil has antisporulant effects against *G. bidwellii* on grape seedling leaves, both against the formation of pycnidia and the production of conidia within pycnidia (Hoffman and Wilcox 2002).

Appendix 8. Banana bract mosaic virus

Scientific name

Banana bract mosaic virus (BBrMV) [Potyviridae: Potyvirus]

Synonyms

None

Common names

Banana bract mosaic disease, bract mosaic disease (Maynaye and Espino 1990), bract mosaic, Kokkan disease (Thomas et al 2000).

Hosts

BBrMV occurs naturally on *M. acuminata* (banana), *M. balbisiana* (Saba banana), *Musa* spp. genotypes AB, AAA, AAB, ABB, ABBB, and *M. textilis* (abacá) (Rodoni et al 1997, 1999; Thomas et al 1997; Sharman et al 2000a). Thomas et al (2000) note that natural hosts of BBrMV are restricted to the genus *Musa* and that no natural sources of resistance are known in *Musa* spp.

Dale (2004) has noted that studies on the host range of BBrMV are not comprehensive.

Muñez (1992) attempted sap transmission tests on *Arachis hypogaea, Carica payaya*, *Chenopodium amaranticolor*, *Cucumis metuliferus, Cucumis sativa*, *Lycopersicon esculentum, Musa* sp., *Nicotiana glutinosa* and *Nicotiana tabacum*, *Passiflora edulis, Passiflora foetida*, *Vigna radiata,Vigna sesquipedalis* and *Zea mays,* without obtaining any BBrMV infections.

Kenyon et al (1997) report that BBrMV was successfully sap-transmitted from bananas to *N. glutinosa, N. tabacum* and subsequently between *Nicotiana* spp. It is noted that Kenyon et al (1997) were unable to transmit the suspected BBrMV from *Nicotiana* spp. back to bananas after 17 trials and cautioned that the virus in *Nicotiana* spp. may not have been BBrMV, because they did not have access to a reliable diagnostic test. There have been no reports of BBrMV occurring naturally in *Nicotiana* spp.

Extensive host range studies have not been reported for BBrMV using aphid vectors. It is also noted that there appear to have been no surveys for BBrMV on weed species in and around banana plantations in the Philippines or elsewhere.

Plant parts affected

All parts of the plants are probably affected. Symptoms have been noted on the leaf lamina and midrib, on the pseudostem, flower bracts and fruit (Magnaye and Espino 1990; Magnaye and Valmayor 1995; Thomas et al 2000). BBrMV has been detected by ELISA, PCR and ISEM in leaf and pseudostem tissue. The virus is transmitted through traditional planting material (corm pieces and suckers) and through micropropagation (Diekmann and Putter 1996). Therefore, it must also be present in the axillary buds of the corm of infected plants.

In the Philippines, a high incidence of BBrMV in commercial Cavendish banana plantations has been correlated with a high rejection rate for malformed bunches and low hand-class ratings (Thomas 1993). Yield losses of up to 40% have been reported in Saba and Cardaba cooking bananas (Roperos and Magnaye 1991).

Distribution

Table 8.1 lists the countries where BBrMV has been recorded.

Table 8.1 Distribution list for Banana bract mosaic virus

Country	References
India	Rodoni et al (1997); Thomas et al (1997)
Philippines	Magnaye and Espino (1990); Bateson and Dale (1995); Thomas et al (1997)
Sri Lanka	Thomas et al (1997)
Thailand	Rodoni et al (1999)
Vietnam	Rodoni et al (1999)
Western Samoa	Rodoni et al (1999)

In the Philippines, BBrMV can be found in almost all smallholdings where Saba and Cardaba cooking bananas are grown (Magnaye and Espino 1990) and is widespread in the plantations from which Cavendish bananas are to be exported (Thomas 1993; Magnaye and Valmayor 1995; Thomas et al 2000).

BBrMV is not known to occur in Australia.

Biology

BBrMV is an obligate parasite that exists as flexuous filamentous particles within living host tissues (Bateson and Dale 1995). The process of establishment, multiplication and movement of BBrMV within host tissues has not been studied but, by analogy to other potyviruses (Shukla et al 1994; Hull 2002), it would be expected that BBrMV would initially replicate in cells at the point of introduction and with the aid of a virus-encoded movement protein, later move through the phloem to a metabolic sink where it would affect subsequent cell development.

Symptoms

Systemic infection by BBrMV results in mosaic patterns in affected tissues (Magnaye and Espino 1990; Thomas et al 2000). The symptoms in floral bracts are diagnostic and distinct from symptoms caused by all other known viruses of bananas. Mosaic patterns, stripes and spindle-shaped streaks may also be visible on pseudostem bases when the outer leaf sheaths are removed and can extend up the petiole bases. Greenish to brownish spindle-shaped streaks may be present on peduncles. Suckering can be suppressed by BBrMV infection and suckers that do emerge can be distorted and deeply pigmented (Thomas et al 2000).

BBrMV can distort the fruit on Cavendish banana bunches (Thomas 1993). Bunches on affected plants can be compact and short, while fingers do not develop well and fruit blemishes can occur (Magnaye and Valmayor 1995). Symptom severity depends on the stage of fruit development when the plant is infected; bunches infected at three weeks of age may have spindle-shaped brown streaks and be so distorted as to be unsaleable, while those infected at a later stage may have only dark green streaks with little distortion of the fingers. In India, petioles and peduncles of ‘Nendran’ become brittle and fruit is only rarely carried to maturity. If fruit does mature, it is undersized. Mosaics can be seen on the fruit of other cultivars.

The expression of disease symptoms can vary over time. For example, Muñez (1992) reported that three of 80 plants inoculated with viruliferous (virus-carrying) *Pentalonia nigronervosa* (banana aphid) under glasshouse conditions developed initial symptoms between 2–5 weeks after inoculation. The symptoms became progressively more severe on successive leaves over the next few weeks, but then gradually disappeared, even on leaves that had previously displayed symptoms. Symptoms reappeared following a change in environmental conditions or when the plants were induced to produce new suckers. This masking effect appeared to be greater under high temperature and bright light than under lower temperature or more shaded conditions, such as occur during the rainy season in the Philippines. Kenyon et al (1997) commented on the occurrence of plants mildly affected with BBrMV in a plot of Lakatan bananas planted near Davao. They also noted that diseased plants were most obvious from January–May and apparently absent from September to December in two years (1996 and 1997).

The symptoms displayed by BBrMV infection can be modified when plants are also infected with other viruses or possibly when mild strains of BBrMV occur. In Western Samoa, India (Tamil Nadu and Maharashtra States) and Vietnam, BBrMV has been isolated from banana plants that were showing symptoms typical of banana mosaic, caused by cucumber mosaic virus (CMV) and lacking the characteristic symptoms on the bracts (Rodoni et al 1997; Caruana and Galzi 1998). Some of these plants were shown to have a mixed infection of CMV and BBrMV.

The masking of symptoms by environmental conditions has been recognised as having implications, both in the selection of mother plant material for micropropagation and for the success of control programs based on inspection and destruction of diseased plants. Thomas (1993) reported that plantlets produced by micropropagation techniques were extensively used in export Cavendish plantations after this technology was introduced into commercial practice over the previous decade. Selections of healthy mother plant material have been based traditionally on visual inspection for freedom from disease symptoms. Although virus-indexing techniques have improved over the past decade (Thomas et al 1997; Rodoni et al 1999; Sharman et al 2000b), they are not widely used in commercial banana propagation. A multiplex immunocapture PCR test is now available for use in the detection of BBrMV in propagation material (Sharman et al 2000b).

Some stakeholders have claimed that the careful selection of planting material, combined with continued field surveillance and eradication, has contributed to a decline in the incidence of BBrMV, to the point that it is now rarely found in export Cavendish plantations. Whether or not there has been a selection of mild or symptomless strains of BBrMV, as suggested by Dale (2004), has yet to be established.

The situation is quite different in smallholder plantations, where BBrMV has continued to develop in epidemic proportions (Thomas and Magnaye 1996). The smallholder plantations are not based on Cavendish bananas, but on varieties such as Saba (ABB), Cardaba (BBB) and Lakatan (AAA), which are preferred in the domestic markets of the Philippines. Kenyon et al (1997) reports that smallholders had a poor appreciation of disease identification, or the need for using clean planting material and prompt removal of diseased plants. There has been little use made of virus-indexed micropropagated plantlets, although there are current Philippine government initiatives to encourage their use.

Transmission

Vegetative transmission

Thomas et al (2000) report that BBrMV can be transmitted through vegetative planting material (including suckers, bits and corms) and through micropropagated plantlets.

Sap transmission

The question of whether or not BBrMV is sap-transmitted is unresolved.

Banana to banana: Thomas et al (2000) report that occasional sap transmission has been achieved. Herradura and Magnaye (1998) report that BBrMV isolates from different parts of the Philippines were transmitted to different *Musa* spp. by sap inoculation, but provide no details of the experiments. Kenyon et al (1997) report sap transmission from banana to banana at a rate of 1 case in 23 trials. Muñez (1992) was unable to transmit BBrMV from banana to banana (no cases in 20 trials).

Banana to herbaceous hosts: Magnaye and Espino (1990) and Muñez (1992) report that attempts to transmit BBrMV by sap inoculation were unsuccessful. Kenyon et al (1997) report that BBrMV was successfully sap-transmitted from banana to *N. tabacum* and *N. glutinosa* and subsequently between *Nicotiana* spp. It is noted that Kenyon et al (1997) were unable to transmit the suspected BBrMV from *Nicotiana* spp. back to banana after 17 trials and cautioned that the virus in *Nicotiana* may not have been BBrMV, because they did not have access to a reliable diagnostic test.

Systemic infection of the Australian native plant, *Nicotiana benthemiana,* has been reported but neither the source of BBrMV nor the method of confirming BBrMV are known.

Aphid transmission

Thomas et al (2000) reported that BBrMV is transmitted in a non-persistent manner by the aphid species *Aphis gossypii, Rhopalosiphum maidis* and *P. nigronervosa*. This report is supported by studies in the Philippines as indicated below.

Magnaye and Espino (1990) reported that BBrMV was transmitted by *A. gossypii* and *R. maidis.* The source of BBrMV in this instance was infected Canara and Morado banana plants, while the test plants were Cavendish bananas. The report indicates there were eight successful transmissions in 20 trials, but provides no details of the numbers of aphids used per test plant, whether they were starved, the numbers of test plants inoculated, or what the exposure time was. No confirmatory tests were undertaken to identify the virus involved.

Muñez (1992) reported that BBrMV was transmitted by *P. nigronervosa*. The source of BBrMV in this instance was leaf tissue from diseased Giant Cavendish plants that had been transplanted from the field to glasshouse pots. The aphids were initially transferred from colonies on healthy Dwarf Cavendish plantlets to moistened filter paper for one hour, presumably to promote probing behaviour, and then placed on infected leaf pieces for periods of 5 seconds, 10 seconds, 60 seconds, 24 hours and 48 hours. After the virus acquisition period, the aphids were transferred in groups of 7 to each of 10 healthy Dwarf Cavendish plantlets for periods of one or seven days. One successful transmission was obtained each with groups of aphids that had a 60 second acquisition feeding time (AFT) and a 24 hours inoculation feeding time (IFT), 1 hour AFT/1 day IFT and 1 hour AFT/7 day IFT. No transmissions were obtained with an AFT of 5 seconds, 10 seconds or 48 hours although, given the small scale of the experiments, such transmission may have been missed by chance. Muñez (1992) interpreted the results as indicating a transmission rate of 10% but, after adjusting for the number of plants per test and the number of aphids used per test plant (Gibbs and Gower 1960), the transmission efficiency of a single aphid was found to average 1.15%. Muñez (1992) found flexuous rod-shaped particles in partially-purified extracts from infected plants that resembled those of a potyvirus.

Kenyon et al (1997) found that BBrMV was transmitted by both *A. gossypii* and *P. nigronervosa*. In these experiments, groups of 10–20 aphids were starved for 3–4 hours before being provided with an AFT of 15–30 minutes followed by an IFT of 24–48 hours. In each experiment, from 1–6 plants of Lakatan, Butuhan or Grand Naine (AAA) banana, or abacá, were inoculated and the number of diseased plants was assessed three weeks after inoculation. Kenyon et al (1997) interpreted the results as indicating a transmission rate in the order of 12–26% but, after adjusting for the effects of numbers of inoculated plants and aphids used per test (Gibbs and Gower 1960), the transmission rates for *A. gossypii* and *P. nigronervosa* averaged 1.25% and 2.69%, respectively.

Herradura et al (2003) found that BBrMV was transmitted by *Pentalonia nigronervosa, A. gossypii* and *R. maidis* but their ability to transmit strains of BBrMV varied. There were three strains of BBrMV used in the study, two from the Philippines and one from Malaysia. *P. nigronervosa* transmitted all three strains tested to *M. acuminata* and *M. balbisiana* equally. After correcting for the effects of aphid numbers per test plant (Gibbs and Gower 1960) the transmission efficiency of individual aphid vectors was estimated to be 1.7–6.7%. *Rhopalosipham maidis* transmitted only one of the three BBrMV strains with an efficiency of 1.7% and then only to the host from which the strain was derived. *Aphis gossypii* transmitted two of the three BBrMV strains to *M. acuminata* with a transmission efficiency of 3.9%. However, it did not transmit any BBrMV to *M. balbisiana.* When Lakatan banana plants were inoculated with 1, 5, 10, 15 or 20 individuals of *P. nigronervosa* at a time, the transmission efficiency was estimated to average 2.8% independently of the aphid number after using the Gibbs and Gower (1960) correction factor. However, the incubation period of the disease was 37 days when five aphids were used, compared with 15 days when 20 aphids were used. No transmissions were obtained when only one aphid was used per test, although this is not surprising given that only 10 test plants were used in each experiment.

Herradura et al (2003) and Muñez (1992) found the efficiency of BBrMV transmission decreased when the IFT exceeded 12 hours. When aphids were given an IFT of 6 and 12 hours, the transmission efficiency of individual aphids was 6.7%, while it was only 2.2% when the IFT was 24 or 48 hours.

This is interpreted as indicating that virus initially acquired during the probing period is inactivated when *P. nigronervosa* settles to feed.

It would appear from the results of transmission experiments for all three species above that an aphid might transmit BBrMV with an efficiency of 1.5–6.7%. However, the results of these experiments involve a combination of both acquisition of BBrMV from an infected host and its subsequent transfer to a non-infected host. If each of these steps is given equal significance, then the efficiencies of acquisition and transfer would each have values of 12–26%.

There appear to be no reports of aphids probing the surfaces of banana fruit or any direct evidence that banana fruit would be a source of virus for aphid vectors. It is to be noted, however, that other fruit can be a source of potyviruses.

Labonne and Quiot (2001) induced apterous *Myzus persicae* to probe apricot fruit infected with plum pox virus and then feed on peach test seedlings. The virus was acquired readily from both immature and over-ripe fruit and transmitted successfully to the test plants.

Lecoq et al (2003) placed yellow cucumber fruit infected with papaya ringspot virus in the centre of field plots of melon trap plants. They observed that, soon after the infected cucumber fruit were set in the field, alates of a range of aphid species probed the fruit surface. They also found that virus infection occurred in trap plants at least 50 m from the virus source, even though the trap plants had been returned to the laboratory for incubation after only 24 hours exposure in the field.

Although the above data on BBrMV transmission are sparse, it appears reasonable to conclude that BBrMV like other potyviruses (Shukla et al 1994; Hull 2002) is readily transmitted through vegetative material and less readily through the probing activities of a range of aphid vectors. It does not appear to be readily sap-transmitted, although this may be achieved occasionally (Thomas et al 2000).

It is also reasonable to conclude, from Muñez (1992) and Kenyon et al (1997), and from analogy with other potyviruses (Shukla et al 1994; Hull 2002), that aphid transmission occurs in a non-persistent manner. It would be expected that virus acquisition would occur during a short period of probing through the cuticle into the epidermal cells of a discarded banana skin and that transmission to other plants would occur during similarly short periods of probing, for a limited time after virus acquisition. A short period is equivalent to a few minutes.

Aphid vectors would be expected to retain a potyvirus such as BBrMV on their stylets or foreguts for a period of minutes to a few hours, depending on the species of aphid, host or strain of virus, and on environmental conditions. The retention time would not be expected to exceed more than a few days (probably less) for any aphid vector of BBrMV. The ability to transmit BBrMV would be lost once an aphid settled to feed on a host plant, although it may be regained if the aphid resumed probing after a period of not feeding.

Aphid biology

General considerations

For an aphid to successfully transmit a plant virus, it must first have access to a plant infected with a virus, then acquire an appropriate dose of the virus, seek out a host plant not already infected with that virus and finally, inoculate the virus into that host plant.

Klingauf (1987) notes that aphids that must find alternative hosts as a result of seasonal change or host maturity may show strong migratory tendencies, while those that do not have to move between host plants show little tendency to migrate. The tendency to migrate varies with the developmental stage of the aphid, being more evident with a young adult alate than with an older alate or an apterous individual. Aphids also undergo behavioural changes before migration, during migration and in the period immediately after migration that alter their preference for walking, flying or settling on a host. There are periods in flight when an aphid will actively seek a host, while at other times it will be carried passively in air currents (often over great distances).

The process of alighting on a surface relies on visual stimuli, including the colour and shape of the host surface. After alighting, aphids walk and probe, responding to physical, olfactory and gustatory stimuli. In response to positive stimuli, aphids increase the frequency and duration of the probing, and the number of pauses. In contrast, on non-hosts they walk more and pause less and the duration of probes decreases.

Pentalonia nigronervosa Coquerel (banana aphid)

Hosts and distribution

The banana aphid occurs naturally on *Musa* spp. and other plant species in the family Musaceae. Species in several closely related plant families including the Araceae (*Alocasia* sp., *Calladium* spp., *Dieffenbachia* spp., *Xanthosma* sp.), Cannaceae (*Canna* spp.), Heliconiaceae (*Heliconia* spp.), Strelitzeaceae (*Strelitzia* spp.) and Zingiberaceae (*Alpinia* spp., *Costus* sp., *Hedychium* spp.) are also colonised by the banana aphid (Wardlaw 1961). However, a degree of host preference is displayed and some difficulty can be experienced transferring them between host species.

The banana aphid has a wide distribution in areas where bananas are grown and is the dominant aphid species found in commercial banana plantations. In Australia, it is found in banana growing areas in Queensland, north-eastern New South Wales, the Northern Territory and the northwest of Western Australia. It has not been recorded in Perth or confirmed in South Australia, Victoria or Tasmania.

Biology

Colonies of aphids can be dense and composed of both alate (winged) and apterous (wingless) forms. The banana aphid does not produce males and offspring are produced by adult females parthenogenetically. The nymphs pass through at least four moults before reaching adulthood. An individual may live for a period of 20–30 days, depending in part on prevailing temperatures (Blackman and Eastop 1984).

On *Musa* spp., banana aphids are found at the base of the pseudostem at soil level and for several centimetres below the soil surface beneath the outer leaf sheaths, at the apex of the pseudostem where new leaf tissue emerges, and under the floral bracts (Magee 1927). They can also be found on fruit when populations are high. Here, the honeydew they produce results in infestations of sooty mould, which is both unsightly and hard to remove (Pinese and Piper 1994).

Magee (1964) summarised unpublished observations on the behaviour of banana aphids under glasshouse and field conditions in New South Wales, which indicated:

- The aphids are more prevalent in the summer than in the colder months, but the colonies persist throughout the year.
- A strong gregarious habit where young nymphs feed very close to the adult parent and form colonies whose boundaries expand gradually under the influence of crowding, with an instinct to reconstitute these colonies after disturbance.
- A negative phototropism in apterous aphids that leads to colony formation in shaded or protected environments in an attempt to avoid light.
- A delay of 4–5 weeks in the appearance of winged nymphs in a new colony with only two to eight individuals with wings per colony.
- A small number of winged adults in young colonies relative to the number of wingless adults, probably due to crowding.
- A tendency of the first winged adults in a new colony to feed *in situ* until there are many winged adults in the colony, whereupon the adult winged aphids become prone to wandering over the leaf surface.
- A tendency for winged adults to fly for a short distance towards bright light in a glasshouse, but after having landed on a glass window, a tendency to wander on this surface for a considerable time.
- Many generations and several weeks pass following initial colonisation before alates occur and migration can take place.
- Although some ants tend some aphids, they have never been observed carrying a wingless individual, nor have been experimentally induced to do so.
- The winged form is reluctant to fly and not capable of prolonged flight.
- Under glasshouse conditions at some times of the year, the distance flown is limited to a couple of metres, at others, a sustained flight of at least six metres has been observed.

Magee (1964) also noted in studies of BBTV that temperatures of 10–15 °C, by retarding the bodily activities of infective aphids and their inclination to feed, reduced the number of successful inoculations under experimental conditions. This may be of some importance in determining the low winter incidence of disease. Aphids not inclined to feed did not produce offspring. Wu and Su (1990b) provided confirmation of the temperature sensitivity of banana aphids.

While aphid feeding and reproductive activity may be slowed by low temperatures, such as those prevailing during handling and transport of green banana fruit, there is no evidence to indicate that low temperatures are lethal to banana aphids. One live banana aphid has been intercepted in Perth on hard green banana fruit from eastern Australia after three days in transit at 13 °C, but there is no evidence of living banana aphids being found on ripened bananas after a further 10–14 days in storage and ripening rooms. The inhibition of reproduction at low temperatures, combined with the ageing of adult aphids would mean that any banana aphids on fruit would likely die during the 14–20 days' transport and handling process.

The period that banana aphids engage in walking and probing behaviour for before settling to feed on abacá can range from 4–24 hours under field conditions (Facundo and Sumalde 1998). The reasons for this variation are unclear but it is evident that, should this occur also on Cavendish bananas, the extended period of walking and probing may favour the ability of the banana aphid to vector BBrMV.

Population and activity

There appear to be no published estimates of the population of banana aphids under field conditions in Australia. In the Philippines, Kenyon et al (1997) used sticky traps to estimate variations in the population (more strictly, the activity) of the banana aphid in or beside a banana plantation. These aphids were trapped in daylight hours, especially in the morning hours and when traps were positioned within one metre of the soil surface. Four to five times more aphids were caught with yellow and pale green sticky traps, than with dark green or brown traps. The numbers of alates caught on yellow sticky traps, each 1.3 m^2 in area and located at the edge of a banana plantation infested with banana aphids (L Kenyon, Plant Virologist/Pathologist, Natural Resources Institute, University of Greenwich, Kent, UK, pers comm 13 February 2006), ranged on average from 0.3–1.25 per month.

Using the data from Kenyon et al (1997) over a five day period, the likelihood of one alate aphid being attracted to a sticky trap is of the order of 0.05–0.21. More insects were caught in yellow and green traps than in other coloured traps. The area of the sticky traps used was approximately 70 times the surface area of waste material from a banana finger. If it is assumed that the yellow colour of the trap simulates the peel from a cluster of discarded fruit in terms of attractiveness to alate aphids, then this data provides an indication of the numbers of banana aphids that might be involved in transmission of BBrMV from fruit waste.

If the discarded waste remains green or yellow for a maximum of five days, for example, it might be expected that the likelihood of one aphid being attracted to the waste from a single banana finger, taking into account the relative size of the waste and the potential to target yellow, would be in the order of 10% of the values cited above or 5.0E–03 to 2.1E–02. The likelihood of an aphid then making a second flight to a suitable host plant has not been quantified, but is not expected to be greater than that given above.

It is noted that the estimates of aphid activity made by Kenyon et al (1997) were obtained in a plantation with 1100 Lakatan banana plants per hectare. It would be expected that the presence of a large number of host plants in proximity to the sticky traps would have influenced the numbers of aphids caught. It is apparent that, while the estimate has relevance to aphid activity in a commercial banana plantation, it would significantly overestimate aphid activity in home garden plantings or other plant communities where plant densities are between 0.005 and 130 mats/km^2 (refer to Table 7.6 in Chapter 7, Part B).

Dispersal

The flight distance of the banana aphid is considered to be relatively short (Magee 1964; Allen 1987; Kenyon et al 1997). In New South Wales, Australia, Allen (1987) found that the dispersal of BBTV by banana aphids could be described by a negative exponential function characterised by a mean spread distance of 15.2 m. In the datasheet for BBTV it is assumed that all but 1% of vectoring flights occur within 70 m.

There is an important difference between BBTV and BBrMV.

- BBTV is transmitted in a persistent manner by the banana aphid and infectivity is retained even if the vector probes non-host surfaces before settling to feed. The mean distance estimated by Allen (1987) represents the total of several intermediate flights before the vector settles to feed.
- BBrMV is transmitted in a non-persistent manner and some infectivity is lost each time a vector probes a non-host surface. The average distance of intermediate flights has not been measured but, if it is assumed that there might be 5–6 flights before an aphid settles to feed, the average flight distance may be in the order of 3 m. If it is further assumed that no more than two flights are made before infectivity is lost, then 99% of aphid flights would be within 30 m.

Aphis gossypii Glover (melon aphid)

The name *A. gossypii* represents an unresolved species complex that exhibits considerable genetic diversity in relation to host plant specialisation, susceptibility to insecticides, parasitism and population adaptation (Fuller et al 1999).

The species is widespread in tropical and temperate areas of the world, including the Philippines and Australia. Although extremely polyphagous in relation to its host range, the melon aphid is a particular pest of cucurbit vegetables, cotton and citrus. It is not considered a pest of bananas (Ebert and Cartwright 1997).

Aphis gossypii can reproduce both sexually and parthenogenetically. In cool temperate areas, the species can overwinter as eggs. Female nymphs hatch in the spring and may feed, mature and reproduce parthenogenetically all summer. In tropical and subtropical areas, parthenogenetic reproduction may occur throughout the year. Nymphs are mainly apterous, but alates are produced when colonies become overcrowded.

The development and fecundity of melon aphids varies considerably between hosts and geographic areas and is strongly affected by the quality of nutrition provided by the host, environmental factors, parasites and predators. At 25 °C on cotton for example, the development time from nymph to adult was approximately six days and 38 nymphs were produced per adult female over a lifespan of 20 days. On other hosts under the same conditions, the production of nymphs varied from 20–64 per adult female over the same time period (Satar et al 1999). Development times may be doubled at 15 °C (Soglia et al 2002).

It appears unlikely that melon aphids would survive the handling and transport process on imported green bananas. Firstly, this aphid is not a pest of bananas, so any aphid on the fruit would be a contaminant not well-adapted to feeding on green banana fruit. Secondly, as it is considered very unlikely that contaminant melon aphids would feed on green banana fruit during transport, it is unlikely to reproduce in transit. Thirdly, any melon aphids that contaminate green banana fruit in the Philippines would be nearing the end of their lifespan by the time of their release into the Australian environment two to three weeks after fruit harvest.

The melon aphid is regarded as a good vector of several potyviruses (Shukla et al 1994), of which BBrMV may be an example. While transmission of BBrMV by melon aphids has been demonstrated experimentally (Magnaye and Espino 1990; Kenyon et al 1997), there is no direct evidence that this aphid spreads BBrMV in the field. Circumstantial evidence in Kenyon et al (1997), however, suggests that some spread of BBrMV could have been associated with the movement of melon aphids from an adjacent citrus orchard through a Lakatan banana plantation.

There appears to be no published data from suction trap or sticky trap studies on the populations of melon aphids present in the Australian environment. Gavarra and Eastop (1975) counted 2000 specimens of melon aphids in yellow pan traps near Davao City in the Philippines over a period of 24 months, finding only 29 banana aphids over the same period. From general literature (Blackman and Eastop 1984), it is expected that populations would build on preferred host species and disperse when conditions favour production of alates. Like the banana aphid, the melon aphid is expected to be attracted to yellow and green surfaces, such as those presented by banana fruit waste. Probing of the epidermal tissue might occur for a minute or less, but it is unlikely that melon aphids would settle to feed on banana tissue. Instead, it would most likely move off in search of a host plant. In the absence of data, the dispersal characteristic is assumed to be similar to that of banana aphids (Figure 9.3).

Rhopalosiphum maidis *Fitch (corn aphid)*

The following data on *R. maidis* is drawn from general literature such as Mau and Kessing (2005).

This aphid species feeds primarily on grasses (Gramineae) and reaches pest proportions towards the end of some growing seasons, particularly on corn (maize). Other hosts include other Gramineae such as wheat, barley, millet, sorghum, rice and sugarcane, and also herbaceous plants such as tobacco, green beans, Manila hemp, papaya, potato and squash.

The corn aphid is Asiatic in origin, but now occurs throughout the tropics, subtropics and warmer temperate regions of the world. It does not persist in regions with severe winter climates.

Populations are primarily composed of parthenogenetic females that produce only live young. Males are occasionally found in cool climates. There can be as many as 50 generations a year in tropical climates. Adults can live for 10–20 days, depending on temperature and other conditions. Winged individuals are produced in response to changes in weather, population density and host plant quality. Moist conditions and high population densities have been observed to increase the production of alate aphids.

This aphid infests all parts of plants above ground and dense colonies may be found in the leaf whorls of preferred hosts towards the end of the growing season. Leaves may become encrusted with aphids and wilt. Under severe conditions, leaves will be dry and chlorotic. The corn aphid excretes copious amounts of honeydew that attracts attending ants, and provides a medium on which sooty mould can grow.

It remains unclear whether or not corn aphids would survive the handling and transport process on imported green bananas. Firstly, this aphid is not a pest of bananas, so any aphid on the fruit would be a contaminant not well-adapted to feeding on green banana fruit. Secondly, as it is considered very unlikely that contaminant corn aphids would feed on green banana fruit during transport, it is unlikely to reproduce in transit. Thirdly, any corn aphid that contaminates green banana fruit in the Philippines would be nearing the end of its lifespan by the time of release into the Australian environment, two to three weeks after fruit harvest.

The corn aphid is regarded as a good vector of several potyviruses (Shukla et al 1994), of which BBrMV may be an example. However, while transmission of BBrMV by *R. maidis* has been demonstrated under experimental conditions (Magnaye and Espino 1990), there is no direct evidence to suggest that this aphid spreads BBrMV in the field.

There appear to be no published data from suction trap or sticky trap studies on the populations of *R. maidis* present in the Australian environment. Gavarra and Eastop (1975) did not detect *R. maidis* in yellow pan traps over a period of 24 months at Davao City in the Philippines, so it may not be attracted to yellow surfaces. If it did probe the epidermal tissue of banana waste, it is unlikely that *R. maidis* would settle to feed. Instead, it would move off in search of a host plant.

Disease incidence in the Philippines

In 1988, the disease reached epidemic proportions around the General Santos City (South Cotabato), where 25,000 mats were destroyed (Magnage and Valmayor 1995). This prompted increased research concerning BBrMV and greater attention to disease control than had been the case, at least in the export-oriented Cavendish banana plantations. Thomas (1993) reported on the incidence of BBrMV-infected plants in 5300 hectares of a group of Cavendish plantations near Davao City that were subject to survey and eradication in the period from 1985–1990 (Figure 8.1). The incidence reached a peak of 2.63 mats per hectare per year in 1987 and decreased progressively to 0.86 mats per hectare per year in 1990. Thomas (1993) reported that the incidence remained relatively constant in the period from 1990 to 1993, with approximately 5000 mats being eradicated each year.

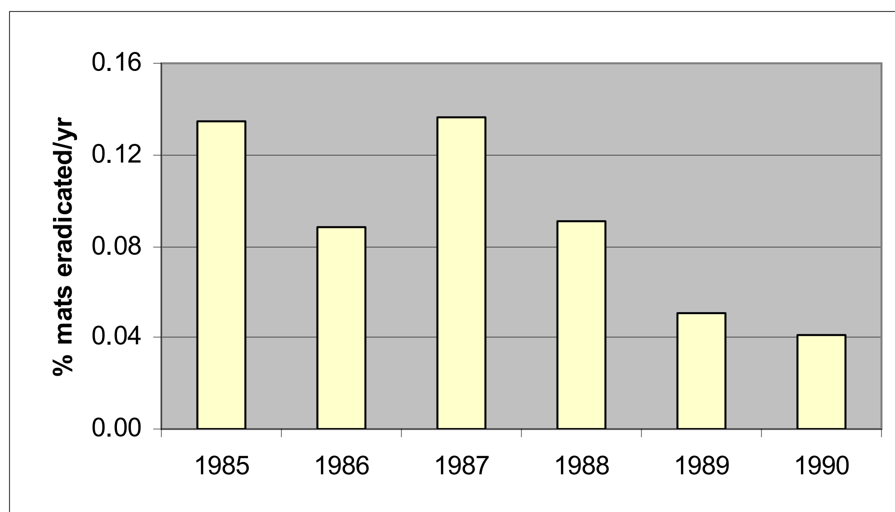


Figure 8.1 Incidence of Banana bract mosaic virus

Mats eradicated each year from a group of Cavendish banana plantations near Davao City. Source: from Thomas (1993)

Given a stated plant density of 2100 mats per hectare (Thomas 1993), these observations suggest that the average proportion of plants with obvious symptoms of BBrMV infection evident at any weekly harvest would be in the range of 7.9E–06 to 2.4E–05. However, the estimates of average incidence obscure a large variation in disease incidence between plantations. Thomas (1993) reported that BBrMV was found in five out of 12 plantations surveyed in 1987 and eight out of 12 surveyed in

1990. Thomas (1993) also reported that only 2000 of the 5300 hectares surveyed were affected by BBrMV in 1990, with 94% of the infections occurring in only two of the 12 plantations surveyed.

Muñez (1992) reported the incidence of BBrMV in one Cavendish banana plantation increased from 1.22 infected mats per hectare per four-week period in early 1989 to 5.26 in late 1990. The proportion of obviously diseased plants at any harvest increased from an average of 1.5E–04 to 6.3E–04 over this 100-week period.

Kenyon et al (1997) reported that the incidence of BBrMV among 600 Lakatan banana plants varied from 0–7 plants per month between January 1996 and September 1997 (Figure 8.2). It was not stated if these plants were subject to weekly inspection and removal of diseased plants as in the Cavendish plantations above but, assuming this is the case, the proportion of plants obviously affected by BBrMV could have been as high as 2.9E–03 at a weekly harvest. Kenyon et al (1997) also noted that there was a strong seasonal pattern in the incidence of BBrMV detection and that, at the end of the observation period in October 1997, there were 100 or more plants with mild symptoms suspected to be associated with BBrMV infection. Such infection could have been a result of spread between plants but could have also reflected a high level of infection in the original planting material that gradually became apparent during the observation period. Virus-indexing technology was not available to test the planting material or plants suspected of being infected at that time (Kenyon et al 1997).

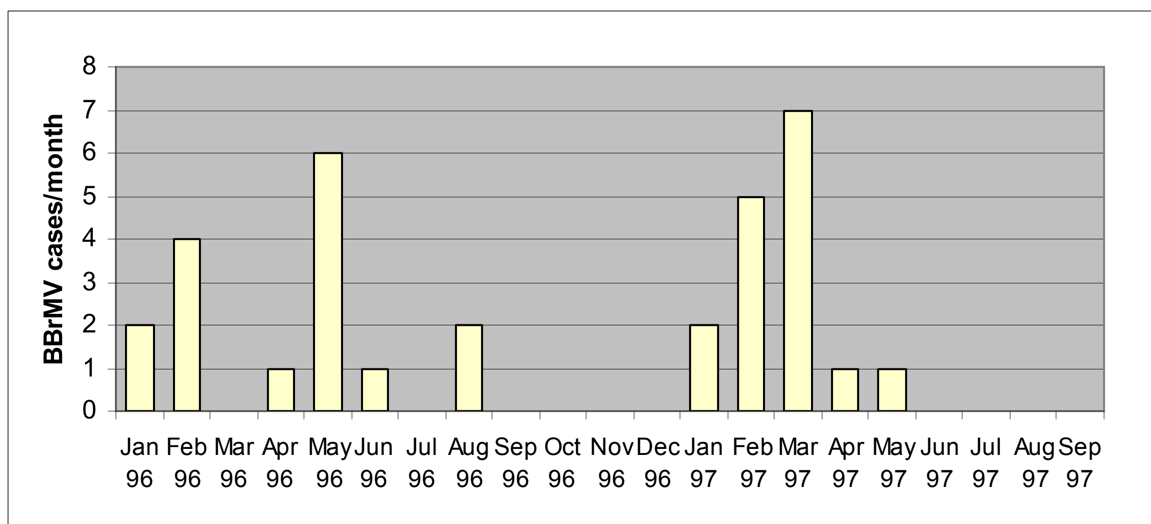


Figure 8.2 Monthly incidence of Banana bract mosaic virus infection

Source: from Kenyon et al (1997), relating to a plot of 600 Lakatan banana plants near Davao City

The current incidence of BBrMV in commercial banana plantations is unclear. While details of recent surveys for BBrMV like those reported by Thomas (1993) have not been published, Philippine authorities have asserted that BBrMV has been encountered only rarely in commercial Cavendish banana plantations since 1997. In contrast with this view, Dale (2004) considers that the evidence from field surveys may underestimate the incidence of infection because the ability to identify infected plants is usually quite specialised. There is good evidence that the symptoms induced by this virus are quite variable and therefore much more difficult to identify. Furthermore, if visible symptoms are the only means used for disease identification, then selection for mild strains of the virus not expressing typical symptoms could be overlooked. The evidence from Kenyon et al (1997) supports the viewpoint of Dale (2004), at least for bananas not derived from virus-indexed mother stock. From Kenyon et al (1997), the incidence of mildly affected plants could be as high as 17%. However, it is difficult to relate this extreme value to the incidence of BBrMV infection under field conditions.

Appendix 9. Banana bunchy top virus

Scientific names

Banana bunchy top virus (BBTV), [Nanoviridae: Babuvirus]

Abacá bunchy top virus (ABTV), [Family: Nanoviridae]. This virus is currently undergoing taxonomic separation from BBTV based on molecular and serological detection assays (J Thomas, Principal Plant Virologist, QDPIF, pers comm 25 July 2006).

Past evidence (Magee 1939; 1953b) indicates that ABTV has epidemiological features similar to BBTV. Its biology has therefore been combined with BBTV in this analysis.

Synonyms

None

Common name

Banana bunchy top disease (Magee 1927; Magnaye and Valmayor 1995; Thomas and Iskra-Caruana 2000).

Hosts

BBTV is known to infect a range of *Musa* species and cultivars in the Eumusa and Australimusa series of edible bananas, as well as *Ensete ventricosum*. Susceptible *Musa* varieties include *M. balbisiana* (Magee 1948; Espino et al 1993), *M. acuminata* subsp. *banksii* and *M. textilis* (abacá) (Magee 1927), *M. velutina* (Thomas and Dietzgen 1991), *M. coccinea, M. jackeyi, M. ornata* and *M. acuminata* subsp. *zebrina* (Thomas and Iskra-Caruana 2000). All hybrid cultivars of *M. acuminata* AA and AAA genomic groups appear to be susceptible, although some hybrids of *M. acuminata* x *M. balbisiana* exhibit mild symptoms or slow disease development (Thomas and Iskra-Caruana 2000). To date, there are no confirmed reports of immunity to BBTV in any *Musa* species or cultivar. However, differences in susceptibility have frequently been noted between cultivars subject to either experimental or field infection (Magee 1948; Jose 1981; Muharam 1984; Espino et al 1993).

There is some evidence for the existence of natural hosts outside the Musaceae, though reports have been conflicting. Su et al (1993) obtained positive ELISA reactions from BBTV-inoculated *Canna indica* and *Hedychium coronarium*, and recovery of the virus to bananas, though not reported here, was demonstrated (Thomas and Iskra-Caruana 2000). Ram and Summanwar (1984) reported *Colocasia esculenta* as a host of BBTV, but Hu et al (1996) were unable to demonstrate *C. esculenta* or *Alpinia purpurata* as experimental or natural hosts of BBTV in Hawaii.

Geering and Thomas (1997) also found no evidence for the following species as experimental (E) or natural (N) hosts of BBTV in Australia: *Alocasia brisbanensis* (E,N), *Alpinia arundinelliana* (E) (taro), *Alpinia caerulea* (E,N) (blue ginger), *Alpinia zerumbet* (E), *C. indica* (E, N), *C.* x *generalis* (N), *C.* x *orchiodes* (N), *Colocasia esculenta* (E,N) (taro), *Strelitzia* sp. (N), *Hedychium coronarium* (E) (ginger flower) and *Heliconia psittacorum* (E) (‘Golden Torch’ heliconia). Magee (1927) was unable to infect *Strelitzia* sp. (bird of paradise), *Ravenala* sp., *Canna* spp. (including *C. edulis*), *Solanum tuberosum* (potato) and *Zea mays* (maize).

Plant parts affected

BBTV infects host plants systemically. It has been detected by ELISA or PCR in most parts of the plant, including leaf lamina and midrib, pseudostem, corm, meristematic tissues, roots, fruit stalk, floral bracts and fruit rind (Thomas 1991; Wu and Su 1990a, 1992; Hafner et al 1995; Geering and Thomas 1997). Disease symptoms are rarely seen on commercially produced banana fruit, although bunches can be distorted in the advanced stages of the disease.

Distribution

BBTV occurs in many, though not all, countries in the south and South-East Asia/Pacific region and various African countries. Significantly, the banana exporting countries of the Latin American/Caribbean region are free from the disease.

The following countries for which there are valid records of banana bunchy top disease have been extracted from Thomas and Iska-Caruana (2000) and Kagy et al (2001) (Table 9.1):

Table 9.1 Distribution list for Banana bunchy top virus

Australia	Guam	Samoa (American)
Burundi	India	Samoa (Western)
Central African Republic	Indonesia	Sri Lanka
China	Japan (Bonin Is., Okinawa)	Taiwan
Congo	Kiribati	Tonga
Democratic Republic of Congo (formerly Zaire)	Malaysia (Sarawak, Malawi)	Tuvalu
Egypt	New Caledonia	USA (Hawaii)
Fiji	Pakistan	Vietnam
Gabon	Philippines	Wallis Is.
	Rwanda	

A strain of BBTV that affects abacá occurs in the Philippines (Ocfemia 1926, 1930) and has been observed in Sri Lanka on both abacá and bananas (Magee 1953b). Reports of BBTV from East Malaysia (Sabah), West Malaysia and Papua New Guinea (Magee 1953a; Wardlaw 1961) need to be authenticated as they were associated with atypical symptoms, were not confirmed by aphid transmission tests and bunchy top of banana was not, and is still not, present.

Philippines

In the Philippines, a high incidence of bunchy top has been observed in the major banana growing areas of northern Luzon, southern Tagalog, western Visayas, and provinces of Mindanao Island (Smith et al 1998; Espino 1999). It is widespread in the southern Mindanao provinces where export Cavendish bananas are grown.

Australia

BBTV occurs in some areas of south-east Queensland south of Cooloolabin, and in the Brunswick and Tweed River valleys of northern New South Wales. It does not occur in Western Australia, the Northern Territory, north Queensland, the Bundaberg area of Queensland, or the Coffs Harbour area of New South Wales (Peasley et al 1998; Thomas and Iskra-Caruana 2000).

Biology

Establishment in a host plant

Plants can become infected with BBTV at any stage of growth (Magee 1927). The virus is introduced by an infective aphid feeding directly on phloem tissue (see aphid transmission below). The virus spreads systemically via the phloem where it affects the differentiation of one or more sieve tubes. It remains in the infected plant tissue until the tissue becomes moribund.

The time course of systemic invasion was studied by Hafner et al (1995), who reported that virion nucleic acids were first detected at seven days, at which time low levels were detected at the inoculation site and in the pseudostem. From 14–41 days after inoculation, virion sense nucleic acid was detected in the pseudostem, corm, roots and new leaf tissue. Virion sense nucleic acids were also detected at the inoculation site and the lower region of the inoculated leaf at 21 days, but not at 14, 31 and 41 days. No hybridisation was observed in extracts from leaves that had already developed prior to inoculation.

Similar results were obtained with complementary sense nucleic acids to BBTV DNA component one, indicating that the virus was replicating in these tissues. Using PCR, BBTV DNA component one was detected at the inoculation site two days after inoculation and apart from its detection in other tissues

previously known to be positive for virion sense nucleic acids, surprisingly it was detected at 21 and 41 days post-inoculation in leaves that had already developed prior to inoculation. This was interpreted as the virus moving to pre-formed tissue in the late stages of systemic spread, but not replicating.

Disease symptoms

The presence of the virus is associated with a profound modification of the structure of the phloem and surrounding tissues of the vascular bundles. The xylem vessels are not affected by the presence of BBTV (Magee 1927, 1939).

Magee (1939) summarised the histological evidence as follows:

> *'The normal phloem tissue is replaced, wholly or in part, by a morbid tissue in which areas of obliteration or necrosis occur. The mechanical sheath of the phloem may be absent or represented by only a few fibres, and the surrounding ground parenchyma is subdivided to form a small-celled tissue of high chloroplast content per given area. It is this chlorophyllous tissue which creates the green streak symptom'.*

He added:

> *'The primary effect of the virus is to cause hypertrophy of cell volume and/or the nucleus of any unspecialised cell adjacent to the sieve tubes and hyperplasia of the more distant cells'.*

The aberrations in the ontogeny of the phloem occur during the incubation period of the disease and subsequently as each leaf or floral bract develops. Increasingly adverse effects are observed with each new leaf or growth cycle. While it is known that bunches may be produced in the early stages of disease development and that these bunches may be distorted (Magee 1927), there appears to be no description of the symptoms or histology of disease in fruit tissue.

The most reliable diagnostic symptom of bunchy top disease in Cavendish bananas and indeed most *Musa* spp. is the characteristic green streaking of veins in the lamina, midrib and petiole of the leaves. Dark red streaks are also evident in the floral bracts. However, on abacá and *E. ventricosum* the effects of infection are restricted to the phloem elements, and the hyperplasia and chloroplast development of the surrounding ground tissue is less intense than in bananas and other *Musa* spp. Symptoms on these plants are characterised by vein-banding and chlorosis, without the green streak symptom characteristic of bunchy top disease in other hosts (Magee 1927, 1953b).

Symptoms become progressively more severe as the degree of phloem derangement intensifies. The leaves of an infected plant become 'bunched' at the apex of the pseudostem as a result of reduced petiole elongation in leaves produced after infection. In its most severe form, bunchy top leads to the leaves becoming upright and progressively shorter, with yellow margins turning necrotic as the leaves age (Thomas and Iskra-Caruana 2000). The most severe symptoms are most commonly seen in plants where the propagation material is derived from a severely diseased plant, or when a plant has been infected for many years (Magee 1927, 1964). All buds on an infected plant become infected with BBTV, which provides an efficient and economically important means of spreading bunchy top infection through vegetative propagation.

Infected plants may produce a bunch in the first growth cycle after infection, with more or less severe symptoms depending on the stage of bunch development when infection occurs. Magee (1927) reported that affected bunches are distorted and rarely saleable. However, there has been no description of disease symptoms on the fruit when the bunch is only mildly affected.

Strain variation

Magee (1953b) presented anecdotal evidence for the existence of mild strains of BBTV. He drew attention to the similarity between the behaviour of BBTV in a clone of Viemama banana (AAA) and that in *M. textilis* and *E. ventricosum.* BBTV infection in these hosts results in symptom expression for an initial period, but they then appear to recover, only to revert to disease expression when new sucker growth is forced. Dark green streaks, characteristic of infection in Cavendish bananas, are not seen on

M. textilis or *E. ventricosum* (Magee 1927, 1939). Mild strains of BBTV, which produce only limited vein clearing and dark green flecks, as well as entirely symptomless strains, have been reported in Cavendish plants from Taiwan (Su et al 1993). Some of these infections may have been caused by the related but taxonomically different abacá bunchy top virus.

At the molecular level, BBTV exists as an icosahedral virion with six complementary, single-stranded DNA genome components (Burns et al 1995). There are significant genetic differences between the strains of BBTV in Australia (the so-called South Pacific isolates) and those that occur in the Philippines, Vietnam and Taiwan (Karan et al 1994; Wanitchakorn et al 2000). Thomas and Iskra-Caruana (2000) report that no biological significance has been attached to these differences, but Dr Dale (JL Dale, Pathologist, School of Life Sciences, QUT, Brisbane, pers comm 4 July 2002) notes there is no information regarding the relative pathogenicity of various virus strains within the two groups or, where they coexist, how this would affect the virulence of viruses through recombination.

The two groups of strains have greater than 10% sequence variation between them – variation within the Philippine group is even greater than between this group and the South Pacific strains.

There is no information regarding the relative pathogenicity of various strains within the two groups or of the possibility of synergism if strains from the two groups were to coexist in a country. Extrapolation from a similar situation in gemini viruses suggests that if heterogenous strains were to coexist in Australia, there would be potential for virus recombination and the generation of increasingly virulent isolates.

Research in Australia has led to the development of transgenic bananas with resistance to the Australian strains of the South Pacific strains. It is unlikely, however, that these transgenic bananas would have cross-protection against the Philippine strains.

Satellite viruses have been found to be associated with the Philippine BBTV strains, which may significantly enhance the virulence of Australian strains of BBTV if they were introduced.

The reported differences between the Asian and South Pacific strains of BBTV, and the observations that infections by the Asian strains may occur at times without displaying disease symptoms, raises significant concerns about the effects of the Asian strains should they be introduced to Australia.

Transmission

Vegetative transmission

Bunchy top is efficiently transmitted through conventional planting material including corms, bits and suckers. All suckers from an infected mat will eventually become infected (Magee 1927). Bunchy top is also transmitted in micropropagated banana plants (Drew et al 1989; Ramos and Zamora 1990; Wu and Su 1991), though not always at rates of 100%. From time to time, apparently virus-free meristems producing apparently virus-free plants can arise from an infected plant (Thomas et al 1995).

Aphid transmission

Magee (1927, 1940) demonstrated that BBTV is transmitted in a persistent manner by the banana aphid, *P. nigronervosa*. Magee (1964) summarised transmission experiments conducted under glasshouse conditions using seedlings of *M. acuminata* subsp. *banksii* as test plants. Although this work was never published in full, the essential points (quoted below) have been confirmed in part by other research:

> *'Transmission has been obtained with individuals of both winged and wingless adult forms of the aphid, and with each of the four nymphal stages preceding each of the adult forms. In one series of trials, approximately 46% of 233 individuals of all stages of the aphid fed as nymphs on recently infected plants have transmitted the disease in subsequent trials.' (Magee 1964)*
>
> *'Adult aphids, fed in the adult stages on infected leaves, have transmitted the virus much less frequently than nymphal forms fed on the same inoculum.' (Magee 1964)*

'[BBTV was] transmitted by infective aphids after feeding for 1½–2 hours on susceptible plants. For acquisition of the virus…a minimum of 17 hours is required, and for high frequency of acquisition a feeding period of 24 hours is advisable.' (Magee 1964) 'There is a delay or "waiting" period in the development of infective power by P. nigronervosa after feeding on infected plants. The duration of this period seems to be dependent on the individual, and to vary from a few hours to approximately two days.'

'The position of inoculation by infective aphids on the leaves of young plants does not greatly influence the rate of infection or the incubation period of the disease' (Magee 1964).

'The virus may be retained by individual infective aphids in daily transfers to fresh plants for periods as long as 13 days after removal from infected plants. During this infective period most, but not all of the test plants infested contract the disease, indicating that special requirements besides the mere feeding of infective aphids are necessary for transmission of the disease. Nymphal aphids may carry the virus through their moults' (Magee 1964).

Hu et al (1996), using micro-propagated Cavendish plantlets and five aphids per test plant, found that aphids became infective after a 4-hour acquisition feed; 20% of individual aphids had acquired BBTV after 4 hours feeding, and a maximum of 90% after 24 hours. Of these infective aphids, 27% were able to inoculate a healthy plant after feeding on it for 15 minutes, rising to 80% after feeding for 2 hours. Figure 9.1 compares these rates of acquisition and inoculation.

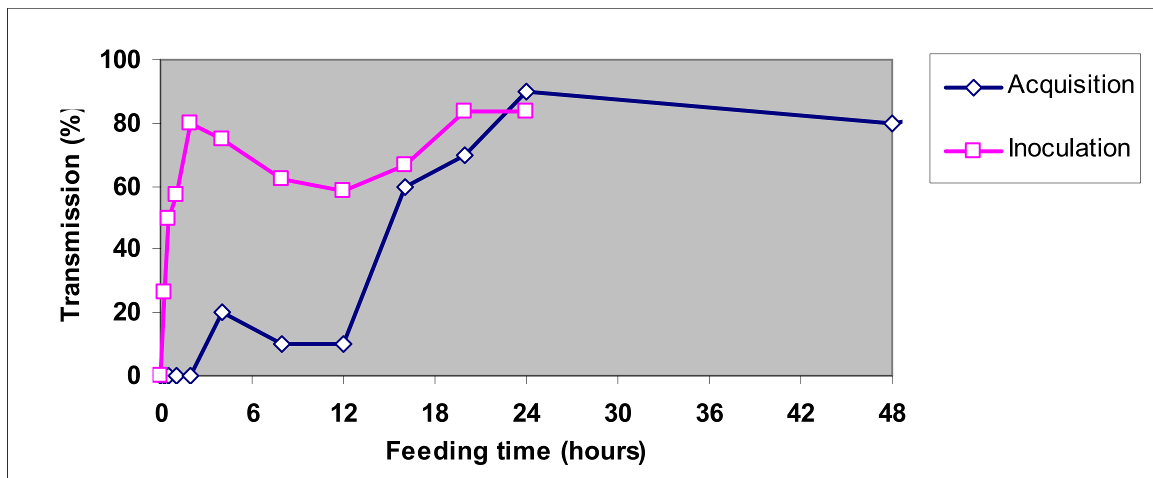


Figure 9.1 Effect of acquisition and inoculation feeding times by *Pentalonia nigronervosa* on the transmission of Banana bunchy top virus

Source: from Hu et al (1996)

Using serial transfers to ginger (demonstrated not to be a host of BBTV), Hu et al (1996) found that BBTV could be detected for the duration of their lives in aphids that had acquired the virus. Up to 80% of the aphids were positive to BBTV by PCR and survived from 10–20 days depending on their age at the beginning of the experiments.

'The virus is not transmitted by infective adults to their viviparous agamic (and only) progeny' (Magee 1964).

Hu et al (1996) transferred 17 adult aphids from an infected banana to ginger plants and, after four days, tested the 17 parents and 131 nymphal progeny using PCR to detect BBTV. Fourteen of the 17 parents were positive for BBTV but all of the progeny were found to be negative.

Hafner et al (1995) demonstrated that BBTV did not replicate in the banana aphid.

'In infected leaves detached from plants and maintained in a fresh condition, the presence of the virus may be demonstrated after a lapse of at least 12 days' (Magee 1964).

'Temperatures of 10 °C and 15 °C, by retarding the bodily activities of infective aphids and their inclination to feed, reduced the number of successful inoculations…under the conditions

of the experiments, and may be of some importance in determining the low winter incidence of disease' (Magee 1964).

Wu and Su (1990b), kept aphids on BBTV-infected plants at temperatures of 16 °C, 20 °C or 27 °C for one month and then transferred them in groups of five to healthy plants held at 20 °C. A transmission rate of 0%, 55% and 100%, was obtained for aphids acquiring BBTV at 16 °C, 20 °C and 27 °C, respectively. However, when aphids that had acquired BBTV at room temperature were fed on healthy plants held at 16 °C for 2–24 hours, all inoculated plants developed bunchy top symptoms. In other words, temperature affected acquisition of BBTV, but not inoculation.

'Infection with bunchy top apparently occurs when a minimal dose of the virus reaches the plant, as an increase in the number of infective aphids during inoculation affects only the frequency of infection and not the severity of symptoms or the minimum incubation period of the disease. Ageing of infected plants, if accompanied as usual by pronounced retardation in growth rate, leads to a pronounced fall in availability of the virus. Such plants may be stimulated to more rapid leaf production by improving the environment, and again rendered highly infectious' (Magee 1964).

Wu and Su (1990b) inoculated one and four-month old banana plantlets by allowing 1–30 infective aphids to feed on each test plant for two days. After adjusting for the statistical effect of the number of aphids per plant (Gibbs and Gower 1960), transmission was found to be reduced in the older plants, but the effect could be overcome by having more infective aphids (Figure 9.2).

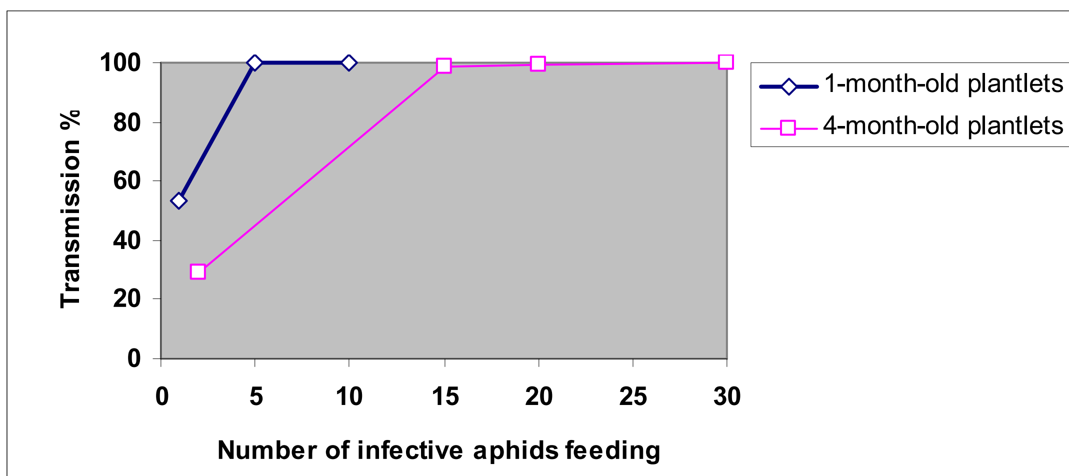


Figure 9.2 Effect of the numbers of inoculating *Pentalonia nigronervosa* on the transmission of Banana bunchy top virus to Cavendish banana plantlets

Source: from Wu and Su (1990b)

Colonies of *P. nigronervosa* from Australia (where bunchy top occurs) and from Réunion Island (where bunchy top does not occur) both transmitted each of six isolates of BBTV with similar efficiency (Thomas and Iskra-Caruana 2000).

Aphid biology

General considerations

For an aphid to successfully transmit a plant virus, it must first have access to a plant infected with a virus, acquire an appropriate dose of the virus, seek out a host plant not already infected with that virus and finally, inoculate the virus into that host plant.

Klingauf (1987) notes that aphids that must find alternative hosts as a result of climate change or host maturity may show strong migratory tendencies, while those that do not have to alternate between host

plants show little tendency to migrate. The tendency to migrate varies with the developmental stage of the aphid, being more evident with a young adult alate than with an older alate or an apterous individual. Aphids also undergo behavioural changes before, during and in the period immediately after migration that alter their preference for walking, flying or settling on a host. There are periods in flight when an aphid will actively seek a host, while at other times it will be carried passively in air currents (often over great distances).

The alighting process involves visual stimuli, including the colour and shape of the host surface. After alighting, aphids display behaviours of walking and probing, responding to physical, olfactory and gustatory stimuli. Positive stimuli usually induce an increase in the frequency and duration of the probing, and the number of pauses on the leaf also increases. In contrast, on non-hosts the duration of successive probes decreases, walking times increase and pauses on the leaf decrease.

Hosts and distribution

The banana aphid (*P. nigronervosa*) occurs naturally on *Musa* spp. and other plant species in the family Musaceae. Species in several closely related plant families including the Araceae (*Alocasia* sp., *Calladium* spp., *Dieffenbachia* spp., *Xanthosma* sp.), Cannaceae (*Canna* spp.), Heliconiaceae (*Heliconia* spp.), Strelitzeaceae (*Strelitzia* spp.) and Zingiberaceae (*Alpinia* spp., *Costus* sp., *Hedychium* spp.) are also colonised by the banana aphid (Wardlaw 1961). However, a degree of host preference is displayed and some difficulty can be experienced transferring them between host species.

Pentalonia nigronervosa has a worldwide distribution in areas where bananas are grown and is the dominant aphid species found in commercial banana plantations. In Australia, it is found in banana growing areas in Queensland, north-eastern New South Wales, the Northern Territory and the northwest of Western Australia. It has not been recorded in Perth or confirmed in South Australia, Victoria or Tasmania.

Colonies of aphids can be dense and composed of both winged alate and wingless apterous forms. *Pentalonia nigronervosa* does not produce males and offspring are produced by adult females parthenogenetically. The nymphs pass through at least four moults before reaching adulthood. An individual may live for a period of 20–30 days, depending in part on prevailing temperatures (Blackman and Eastop 1985).

On *Musa* spp., banana aphids are found at the base of the pseudostem at soil level and for several centimetres below the soil surface beneath the outer leaf sheaths, at the apex of the pseudostem where new leaf tissue emerges, and under the floral bracts (Magee 1927). They can also be found on fruit when populations are high. Here, the honeydew they produce results in infestations of sooty mould, which is both unsightly and hard to remove (Pinese and Piper 1994).

Magee (1964) summarised unpublished observations on the behaviour of *P. nigronervosa* under glasshouse and field conditions in New South Wales, which indicated:

- The aphids are more prevalent in the summer than in the colder months, but the colonies persist throughout the year
- A strong gregarious habit where young nymphs feed very close to the adult parent and form colonies, with boundaries expanding gradually under the influence of crowding and supported by an instinct to reconstitute these colonies after disturbance
- A negative phototropism in apterous aphids that leads to colony formation in shaded or protected environments in an attempt to avoid light.
- A delay of 4–5 weeks in the appearance of winged alates in a new colony with from 2–8 individuals.
- A small number of winged adults in young colonies relative to the number of wingless adults, with crowding likely to be the initiating factor.
- A tendency of the first winged adults in a new colony to feed in situ until there are many winged adults in the colony, whereupon the adult winged aphids become prone to wandering over the leaf surface.
- A tendency for winged adults to fly for a short distance towards bright light in a glasshouse, but

after having landed on a glass window, a tendency to wander on this surface for a considerable time.

- Many generations and several weeks pass following initial colonisation before alates occur and migration can take place.
- Although some ants tend some aphids, they have never been observed carrying a wingless individual, nor have they been experimentally induced to do so.
- The winged form is reluctant to fly and not capable of prolonged flight.
- Under glasshouse conditions at some times of the year, the distance flown is limited to a couple of metres, at others, a sustained flight of at least six metres has been observed.

Magee (1964) also noted in studies of BBTV that temperatures of 10–15 °C, by retarding the bodily activities of infective aphids and their inclination to feed, reduced the number of successful inoculations under experimental conditions, and may be of some importance in determining the low winter incidence of disease. Aphids not inclined to feed did not produce offspring. Wu and Su (1990b) provided confirmation of the temperature sensitivity of banana aphids.

While aphid feeding and reproductive activity may be slowed by low temperatures, such as those prevailing during handling and transport of green banana fruit, there is no evidence to indicate that low temperatures are lethal to banana aphids. Live *P. nigronervosa* have been intercepted in Perth on hard green banana fruit from eastern Australia after three days in transit at 12–14 °C (Carnarvon Banana Industry Protection Committee (2004), but there is no evidence of living banana aphids being found on ripened bananas after a further 10–14 days in storage and ripening rooms. The inhibition of reproduction at low temperatures, combined with the ageing of adult aphids, would mean that any banana aphids on fruit would likely die during the 14–20 day transport and handling process.

The period that individuals of *P. nigronervosa* engage in walking and probing behaviour for prior to their settling to feed on abacá can range from 4–24 hours under field conditions (Facundo and Sumalde 1998). The reasons for this variation are unclear but it is evident that, should this also occur on Cavendish bananas, the extended period of walking and probing may not favour the ability of the banana aphid to acquire BBTV from discarded banana waste material.

Population and activity

There appear to be no published estimates of the population of *P. nigronervosa* under field conditions in Australia. In the Philippines, Kenyon et al (1997) used sticky traps to estimate variations in the population (more strictly, the activity) of the banana aphid in or beside a banana plantation. These aphids were trapped in daylight hours, especially in the morning hours and when traps were positioned within one metre of the soil surface. Four to five times more aphids were caught with yellow and pale green sticky traps, than with dark green or brown traps. The numbers of alates caught on yellow sticky traps, each 1.3 m^2 in area and located at the edge of a banana plantation infested with *P. nigronervosa* (L Kenyon, Plant Virologist/Pathologist, Natural Resources Institute, University of Greenwich, Kent, UK, pers comm 13 February 2006), ranged on average from 0.3–1.25 per month.

Using the data from Kenyon et al (1997) over a five day period, the likelihood of one alate aphid being attracted to a sticky trap is of the order of 0.05–0.21. More insects were caught in yellow and green traps than in other coloured traps. The area of the sticky traps used was approximately 70 times the surface area of waste material from a banana finger. If it is assumed that the yellow colour of the trap simulates the peel from a cluster of discarded fruit in terms of attractiveness to alate aphids, then this data provide an indication of the numbers of banana aphids that might be involved in transmission of BBTV from fruit waste.

If the discarded waste remains green or yellow for a maximum of five days, for example, it might be expected that the likelihood of one aphid being attracted to the waste from a single banana finger, taking into account the relative size of the waste and the potential to target yellow, would be in the order of 10% of the values cited above or 5.0E–03 to 2.1E–02. The likelihood of an aphid then making a second flight to a suitable host plant has not been quantified, but is not expected to be greater than that given above.

It is noted that the estimates of aphid activity made by Kenyon et al (1997) were obtained in a plantation with 1100 Lakatan banana plants per hectare. It would be expected that the presence of a large number of host plants in proximity to the sticky traps would have influenced the numbers of aphids caught. It is apparent that, while the estimate has relevance to aphid activity in a commercial banana plantation, it would significantly overestimate aphid activity in home garden plantings or other plant communities where plant densities are between 0.005–130 mats per km^2 (refer to Table 7.6 in Chapter 7, Part B).

Dispersal

The flight distance of *P. nigronervosa* is considered to be relatively short (Magee 1964; Allen 1978b, 1987; Kenyon et al 1997). In New South Wales, Allen (1978b, 1987) found that the dispersal of BBTV by *P. nigronervosa* could be described by a negative exponential function characterised by mean spread distance of 15.2 m. If the extinction point is assumed to be 1% of the dispersing aphid population, the effective range of aphid dispersion is estimated to be 70 m as illustrated in Figure 9.3. Such an extinction point is relevant to dispersal in the special case when a total of 100 aphids are involved, as less than one aphid would be expected to disperse more than 70 m. The range of dispersal would be expected to be proportionally less than 70 m when a small number of aphid vectors are involved.

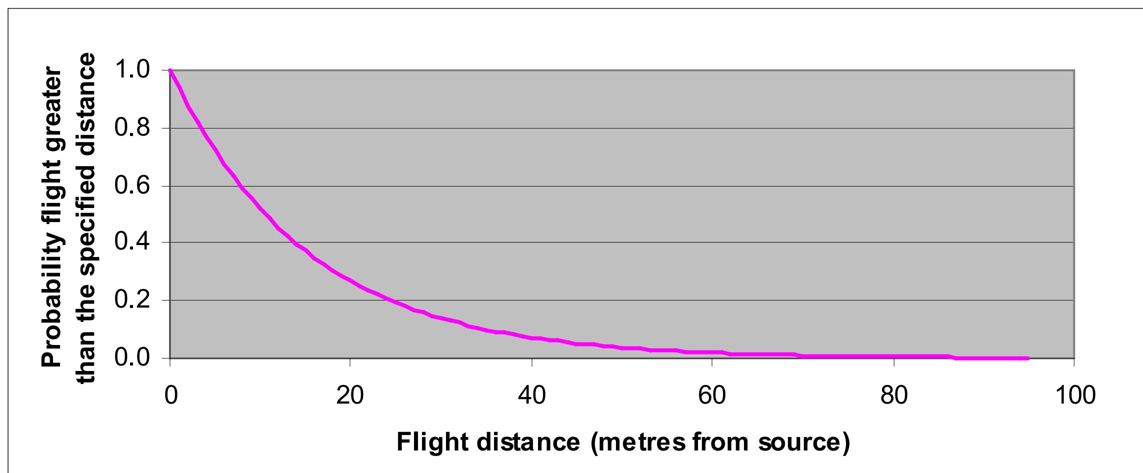


Figure 9.3 Assumed dispersion characteristic for alates of *Pentalonia nigronervosa*

Rate of decline based on a mean spread distance of 15.2 m given in Allen (1987)

BBTV infection rates

The rate at which bunchy top disease spreads in commercial Cavendish banana plantations in New South Wales was estimated by Allen (1978a) to vary from a maximum of 0.027 new infected plants/old infected plant per day in summer, to a minimum of 0.001 new infected plants/old infected plant per day in winter. Allen (1987) subsequently estimated the basic infection rate to vary from a maximum of 0.064 new infections/infectious plant per day in summer to a minimum of 0.0044 new infections/infectious plant per day in winter. It would be expected that the higher rates would apply in tropical areas such as North Queensland.

This estimate of the basic infection rate above represents the apparent activity of aphid vectors moving from infectious plants (that is, those with available virus) to non-infected plants in a situation where the availability of susceptible host plants is non-limiting. In situations where only a few host plants are present, such as in a home garden, the rate would be reduced in proportion to the target area of the host surfaces.

A further factor in applying the estimates of the basic infection rates above is that the aphid vectors were, in the main, moving to healthy plants from established colonies on infected plants. The basic infection rates were therefore indicative of the inoculation aspect of transmission. There are no estimates available on the efficiency of virus acquisition in the field but, given the short time available

and the small target area of waste material compared with that of a banana plant, the efficiency would be several orders of magnitude less than the 80% indicated in Figure 9.1.

It is noted that there is a correspondence between the basic infection rate estimated for BBTV in the summer months of New South Wales (0.32 new infections/infectious plant per 5-day period from Allen (1987)) and the likelihood of an aphid visiting the simulated waste from a cluster of bananas over the same time period (a value of 0.05–0.21 from Kenyon et al (1997)). The differences in these estimates could well reflect the relative sizes of trap or host material.

Incubation and latent periods in Cavendish bananas

The incubation period is the time between inoculation of the plant by an aphid and the appearance of symptoms. Magee (1927) observed that disease symptoms could be found in the first leaf to emerge from the pseudostem after inoculation, but the symptoms were often not displayed until three or more leaves had emerged. An average of 1.7 leaves emerged between inoculation and the appearance of disease symptoms. Allen (1978a) demonstrated that the incubation period was correlated throughout the year with the time for 1.7 new leaves to emerge but varied under subtropical conditions from 19 days in summer to 125 days in winter, mainly as a result of the effects of temperature on leaf emergence rate.

The latent period is the time between inoculation of the plant by an aphid and the development of an infectious condition in the plant, that is, the occurrence of alate aphids that have acquired BBTV from that plant and are ready to spread BBTV to other plants. Allen (1987) demonstrated that the latent period was correlated throughout the year with the time for 3.7 new leaves to emerge. The significance of this observation is that inspections made at intervals of less than the latent period are able to achieve disease control through prompt eradication of diseased plants provided that detection and eradication efficiency are high. Inspection intervals of three weeks have been found to be acceptable in subtropical New South Wales (Eastwood 1946; Allen 1978a), whereas weekly inspection intervals supported by additional eradication measures are necessary in the Philippines.

BBTV incidence in Philippine Cavendish banana plantations

From 1993 to 1996, Smith et al (1998) studied the incidence of BBTV in a 220 hectare area at the edge of a large Cavendish banana plantation near Davao City, the Philippines, which bordered banana plantations heavily infested with BBTV. The study area was subject to a BBTV control program involving inspections every 1–2 weeks, eradication of BBTV plants, and replacement with healthy plants. The highest incidence recorded in the whole area was 0.14% in April 1996, or 3.18 cases per hectare per 4 week period. However, the incidence of BBTV decreased exponentially with increases in distance from the border adjacent to the heavily BBTV-infested bananas, with 97% of BBTV detected within 800 m of the border (Figure 9.4). The incidence in the 100 metre wide block nearest the border was 7 cases per hectare per 4 week period, or 0.32%.

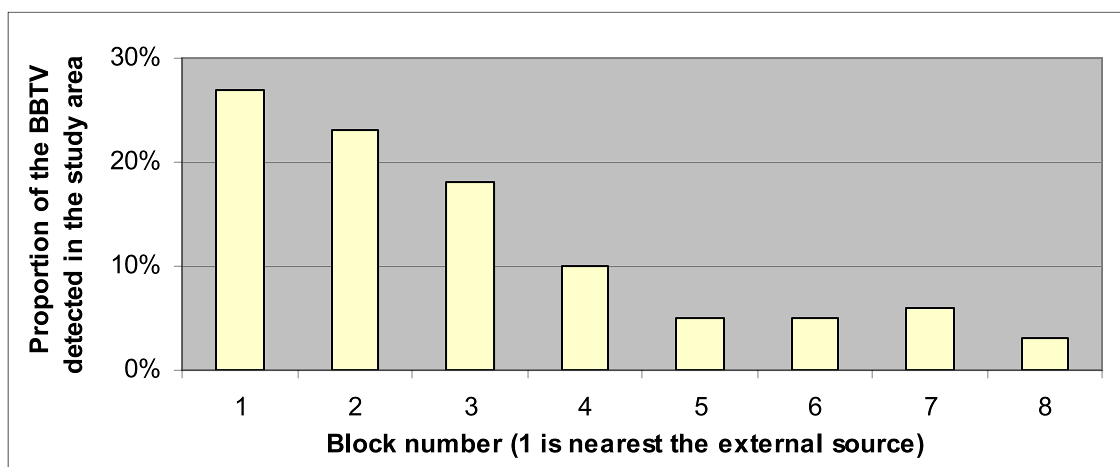


Figure 9.4 Incidence of Banana bunchy top virus by distance from an external source of

Banana bunchy top virus

Source: from Smith et al (1998) relating to 100 m wide blocks of Cavendish bananas

Survey data provided by BPI (BPI 2002a) indicates that in the years from 1998–2001, the incidence of BBTV varied from 0.08–0.471 cases (infected mats) per hectare per 4-week period. The average incidence was 0.185 new cases per hectare per 4-week period and the mode was approximately 0.13 cases per hectare per 4-week period (Figure 9.5). No information has been provided on how these data were derived other than that they were from Cavendish banana plantations from which export fruit was harvested.

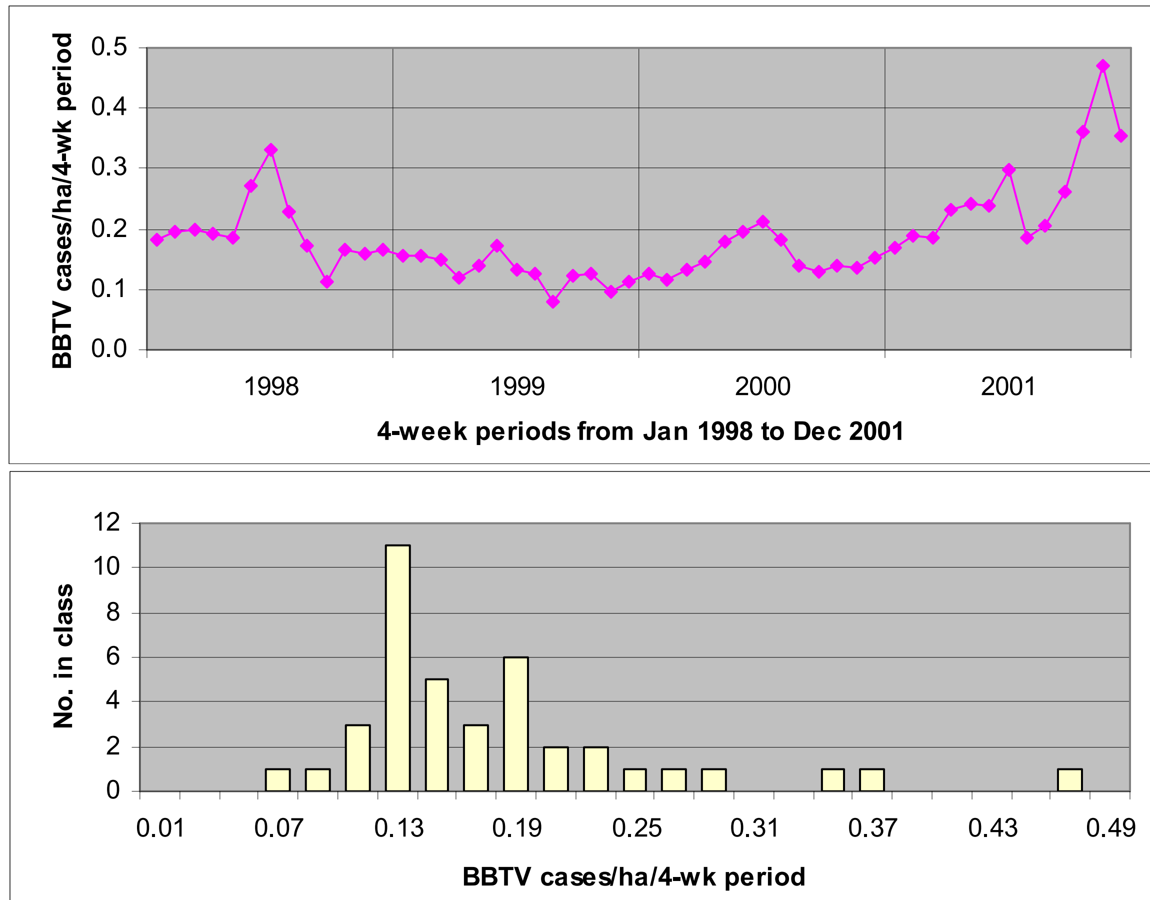


Figure 9.5 Incidence of Banana bunchy top virus cases in Cavendish banana plantations

Data provided by Philippines authorities (BPI 2002a), presented as cases per hectare per four-week period (top) and as a frequency distribution of the incidence data (bottom)

Seasonal trends in disease incidence

Magee (1964) commented that the incidence of bunchy top detected in Australian control operations varied seasonally, with a maximum observed in summer and a minimum in the colder months. Allen (1978a, 1987) demonstrated that this seasonal pattern was correlated primarily with seasonal effects on the incubation period of the disease and some minor seasonal effects on the detection and eradication efficiencies.

Smith et al (1998) examined survey records from 1983–1996 from the whole plantation of which the 200 hectare study area was a part (Figure 9.6). No details were provided on the total area of this plantation, but there was a consistent annual periodicity in BBTV detection with twice as many plants detected in the month of May than in November (Figure 9.6). Disease incidence also increased exponentially over the period of the survey in spite of control measures, being more than 12 times greater in 1996 than 1983 (Figure 9.6).

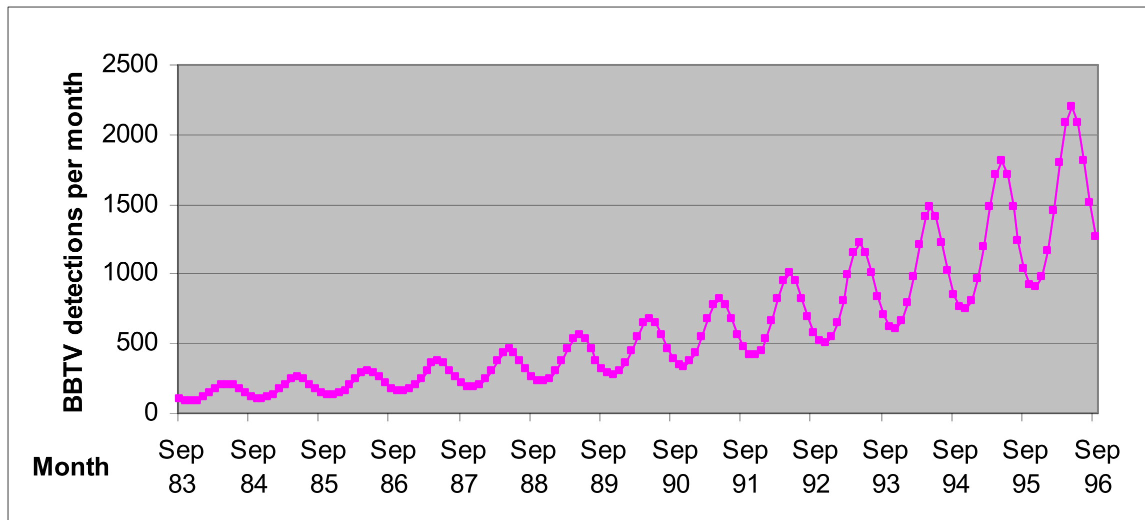


Figure 9.6 Seasonal incidence of Banana bunchy top virus

Source: from Smith et al (1998), relating to a Cavendish banana plantation near Davao City

Impacts of BBTV

BBTV infection affects the productivity of plants to the extent that severely diseased plants do not produce saleable fruit. Mildly affected plants, such as those infected late in the production cycle, may produce normal fruit in which the eating quality is not affected. However, BBTV can be a devastating disease if not controlled (Magee 1927).

The economic impact of bunchy top disease in well-managed banana plantations occurs as a result of the costs associated with control measures required to exclude the disease or to keep disease incidence at low levels. In Australia, quarantine restrictions prohibit the use of BBTV-infected plants for propagation purposes. Technology is available to produce plants from virus-tested mother stock, while the movement of planting material between quarantine areas is regulated. Banana growers also destroy any infected plants that are found by regularly inspecting plants for disease symptoms. Similar control measures are employed in the Philippines, where disease control requires weekly inspections and the destruction of all plants within five metres of any diseased plant.

No quarantine restrictions apply to the marketing of fruit from banana plantations affected by bunchy top disease in Australia. However, fruit from eastern states is inspected for the presence of *P. nigronervosa* on arrival in Western Australia (refer Part C, Appendix 1).

Appendix 10. Fruit flies

Two species of fruit fly are of potential quarantine concern to the whole of Australia and occur on bananas in the Philippines.

Scientific name

Bactrocera occipitalis (Bezzi) [Diptera: Tephritidae]
Bactrocera philippinensis Drew and Hancock [Diptera: Tephritidae]

Note: *B. occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock both belong to the Oriental fruit fly *Bactrocera dorsalis* (Hendel) species complex. They occur in the same geographical area, but do not interbreed (Iwaizumi 2004).

Synonyms

Bactrocera occipitalis: *Chaetodacus ferrugineus var. occipitalis* (Bezzi), *Dacus (Strumeta) dorsalis var. occipitalis* (Bezzi), *Dacus (Strumeta) occipitalis* (Bezzi), *Dacus (Bactrocera) occipitalis* (Bezzi), *Bactrocera (Bactrocera) occipitalis* (Bezzi).

Bactrocera philippinensis: *Dacus (Strumeta) pedestris* Hardy, 1974 (Misidentification), Philippine B. by Drew (1988) and *Bactrocera* sp. nr *B. dorsalis* (C) by White and Elson-Harris (1992), *Bactrocera (Bactrocera) philippinensis* sp. n. (Drew and Hancock 1994).

Both species were previously identified as *Dacus dorsalis* Hendel in the Philippines. Rejesus et al (1988) stated that, depending on taxonomic verification of past identifications, a change would occur to separate fruit fly species that looked very similar. This resulted in the fruit flies being identified as *B. occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock.

Common names

Bactrocera occipitalis: Fruit fly
Bactrocera philippinensis: Philippine fruit fly

Hosts

A host is defined by Cowley et al (1992) as any fruit or vegetable in which fruit flies oviposit under field conditions, eggs hatch into larvae and these larvae acquire sufficient sustenance to form viable pupae, from which adults capable of reproduction emerge. Armstrong (1994) states that this definition of quarantine host overlooks commodities that are resistant to infestation during certain stages of growth or maturity. Some commodities that are hosts for quarantine fruit flies may not be susceptible to infestation during their early stages of maturity, for example, bananas, papaya and limes (Heimoana et al 1997).

The following host lists were compiled from a number of sources, including Allwood et al (1999), Drew and Hancock (1994), Mango Information Network (2005), Pacifly (2002a, 2002b), Rejesus et al (1988), Sengebau et al (2005), Western Micronesia Regional Invasive Species Council (2005a, 2005b) and Ms Obra (G Obra, Senior Science Research Specialist, Atomic Research Division, Philippine Nuclear Research Institute pers comm 2 February 2006).

Bactrocera occipitalis has 22 recorded hosts from 14 plant families: *Anacardium occidentiale* (cashew); *Annona muricata* (soursop); *Annona squamosa* (sugar apple); *Artocarpus* sp. (jackfruit); *Averrhoa carambola* (starfruit, carambola); *Carica papaya* (papaya); *Citrus macrocarpa; Citrus madurensis* (calamondin orange, Philippine lime); *Citrus reticulata* (mandarin); *Citrus* sp.; *Diospyros blancoi* (velvet apple); *Mangifera indica* (mango); *Manilkara zapota* (sapodilla); *Musa acuminata*

(banana, Cavendish variety, ripening and ripe stages); *Passiflora edulis* (passionfruit); *Persea americana* (avocado); *Psidium guajava* (guava); *Sandoricum koetjape* (wild mangosteen, lolly fruit); *Spondias purpurea* (purple mombin); *Syzygium cumini* (jambolan plum); *Syzygium malaccense* (Malay apple); *Ziziphus jujuba* (common jujube).

Bactrocera philippinensis has 24 recorded hosts from 14 plant families: *Anacardium occidentiale* (cashew); *Annona muricata* (soursop); *Annona squamosa* (sugar apple); *Artocarpus altilis* (breadfruit); *Artocarpus communis* (breadfruit); *Averrhoa carambola* (starfruit, carambola); *Carica papaya* (papaya); *Citrofortunella mitis* (kumquat); *Citrus madurensis* (calamondin orange, Philippine lime); *Citrus reticulata* (mandarin); *Citrus* sp.; *Diospyros blancoi* (velvet apple); *Mangifera indica* (mango); *Manilkara zapota* (sapodilla); *Musa acuminata* (banana, Cavendish variety, ripening and ripe stages); *Passiflora edulis* (passionfruit); *Persea Americana* (avocado); *Pouteria duklitan; Psidium guajava* (guava); *Sandoricum koetjape* (wild mangosteen, lolly fruit); *Spondias purpurea* (purple mombin); *Syzygium cumini* (jambolan plum); *Syzygium malaccense* (Malay apple); *Ziziphus jujuba* (common jujube).

Bactrocera philippinensis and *B. occipitalis* attack the same plant families, but *B. occipitalis* is slightly less damaging than the closely related *B. philippinensis* (Western Micronesia Regional Invasive Species Council 2005b).

Plant parts affected

Fruit (Drew and Hancock 1994).

Distribution

Bactrocera occipitalis is native to tropical Asia (Philippines, Brunei and Malaysia – Borneo, Sabah). It was introduced into Palau.

Bactrocera philippinensis is native to the Philippines and has also been introduced into Palau.

Increased fruit fly infestations were detected in Palau in 1995 (Republic of Palau 2002). However, the fruit flies were not officially identified as *B. occipitalis* and *B. philippinensis* until 1996 when they were collected in traps (Pacifly 2004). Introductions into Palau were probably from Asia by travellers who carried infested fruits not declared to quarantine (Pacifly 2004). Sengebau reported (F Sengebau, Head of Plant Protection and Quarantine Service, Bureau of Agriculture, Ministry of Resources and Development, Republic of Palau, pers comm 6 December 2005) that there are a large number of Filipinos living in Palau, making up almost 25% of the population. Therefore, it is highly likely that these fruit flies entered Palau with travellers from the Philippines.

Table 10.1 lists the countries where *B. occipitalis* and *B. philippinensis* have been recorded.

Bactrocera philippinensis was also detected on mainland Australia near Darwin in the Northern Territory in November 1997, but was eradicated by 1999 (Cantrell et al 2002).

Table 10.1 Distribution list for Bactrocera occipitalis and Bactrocera philippinensis

Source: Drew and Hancock (1994), SPC (2001), Pacifly (2002a, 2002b), Sengebau et al (2005) and Western Micronesia Regional Invasive Species Council (2005b).

B. occipitalis	***B. philippinensis***
Brunei	Palau
Malaysia (Borneo, Sabah)	Philippines
Palau	
Philippines	

Biology

These two species are typical dacine fruit flies of the Oriental fruit fly complex. The adult body length is 7–8 mm and the wing length about 6 mm. They have a black thorax with yellow markings, brown abdomen with black markings and transparent wings, with two dark bands on each. The abdomen of *B. occipitalis* is slightly darker than that of *B. philippinensis* (Figure 10.1). Drew and Hancock (1994) provided a detailed description of their diagnostic characteristics.

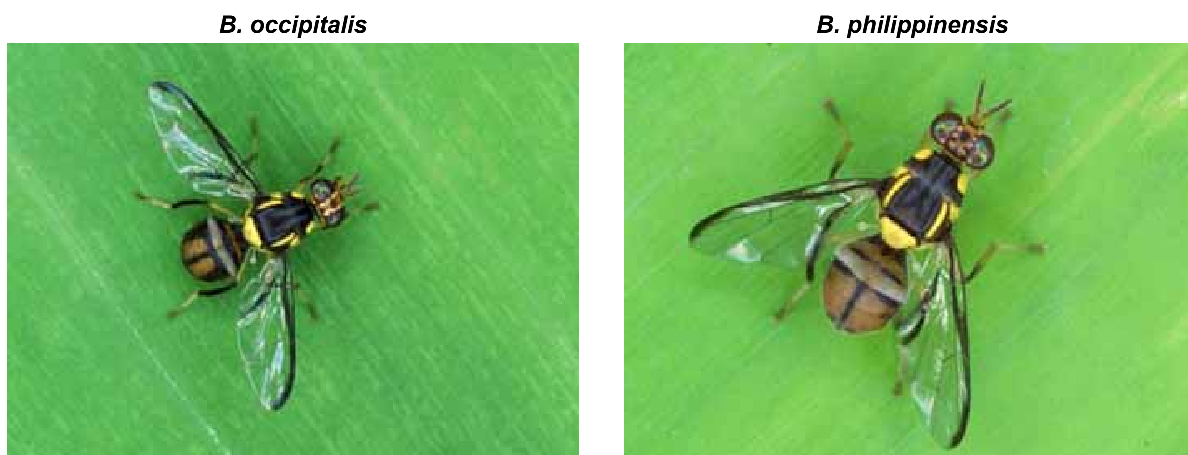


Figure 10.1 ***Bactrocera occipitalis*** **and** ***Bactrocera philippinensis***
Source: © 2002 Secretariat of the Pacific Community and Contributors

Lifecycle in host plants

The following specific biological details for *B. occipitalis* and *B. philippinensis* are from Sengebau et al (2005) and the Western Micronesia Regional Invasive Species Council (2005a, 2005b). Fruit fly activity is centred on the host plant, which provides a site for adult resting or shelter, feeding, mating and egg-laying, as well as larval and pupal development in the soil underneath the plant. Using its egg-laying organ (ovipositor), the adult female lays its eggs in clutches under the skin of the fruit. In the process, the female introduces bacteria into the fruit. The bacteria cause the fruit to break down and rot. One to two days following egg laying, larvae (maggots) hatch out and feed on the decaying fruit flesh. The larvae develop through three larval stages (instars) in about seven days. During this time, fruit often falls to the ground prematurely.

The third instar larvae escape from the fruit and burrow into the soil or organic matter and form yellowish-brown pupae. Pupal development takes about 10 days. Twenty days after the eggs are laid adult flies emerge from the puparium and disperse.

Obra (G Obra, Senior Science Research Specialist, Atomic Research Division, Philippine Nuclear Research Institute, pers comm 8 February 2006) advised that *B. philippinensis* has the following lifecycle in the laboratory when provided with an artificial diet: *Bactrocera philippinensis* eggs hatch in 1–2 days, the larval stages last seven days, followed by a pupal stage of nine days after which the adults emerge. In the laboratory, the lifecycle takes a maximum of 18 days.

Shortly after the females emerge, they disperse in search of a protein meal, essential for producing viable eggs. Adult fruit flies are reasonable fliers.

Although it is possible that *B. philippinensis* may lay eggs under the peel of mature hard green undamaged bananas, it is important to identify whether hard green bananas are a host to *B. philippinensis* (that is, whether *B. philippinensis* eggs will hatch into larvae, and whether these larvae will acquire sufficient sustenance from the mature hard green undamaged bananas to form viable pupae from which adults capable of reproduction will emerge).

An evaluation of the literature, pest interceptions and other evidence can usually determine if a specific commodity at a specific maturity stage is a host for the pest of concern (IAEA 2005).

Armstrong (1983) found that three species of fruit fly, *B. dorsalis, B. cucurbitae* (melon fly) and *Ceratitis capitata* (Mediterranean fruit fly), readily laid eggs into mature green bananas. However, the eggs or the first instar larvae that hatched from them did not survive. Armstrong (1983) found that unripe bananas formed dark, hard tissue around egg laying sites, encapsulating the eggs. Latex was also produced at the site surrounding the eggs and forming a sticky surface onto which eggs and first instar larvae would adhere, leading to their death. As the latex hardened, it formed an adhesive cap over the site, suffocating the eggs and any first instar larvae.

Armstrong (1983, 2001) concluded that banana was not a host for *B. dorsalis, B. cucurbitae* and *C. capitata* when the bananas are hard green, undamaged and attached to the banana plant, or for up to 3–4 days post-harvest. Brown (1998) stated that eggs of most fruit fly species, with the exception of *B. musae* (the banana fruit fly), fail to hatch if laid in hard green bananas.

Based on the above information:

- New Zealand permits the importation of green bananas from Ecuador, Mexico, Niue, the Philippines, Samoa and Tonga (Pacifly 2002b; Biosecurity New Zealand MAF, Wellington, New Zealand 2005c). New Zealand’s draft *Import health standard for bananas from Australia* also accepts bananas to be non-hosts to economically important Australian fruit flies (including *B. musae*) when in the unripe ‘hard green’ maturity stage (Biosecurity New Zealand MAF, Wellington, New Zealand 2006).
- The United States permits imports of green bananas from Africa, the Caribbean, the Pacific region and South America (USDA 2005).

Host status testing of hard green bananas during the *B. papayae* outbreak in Australia in 1995 found that hard green bananas were not hosts to this species of fruit fly (Pinese and De Faveri 1996; Cantrell et al 2002). The work of Pinese and De Faveri (1996) was used to develop an interstate certification assurance (ICA) document to enable Queensland bananas to be exported around Australia (Cantrell et al 2002). ICA–06 *Hard green condition of bananas* describes the operational procedures including interstate fruit fly quarantine conditions, for interstate movement of Queensland bananas. It was selected as the most suitable for bananas from the Philippines, as it requires hard green bananas to conform to the following criteria (QDPI 2000):

The variety is Cavendish only.

- The banana flesh is hard and not flexible. The skin is green and shows no yellow colouration except for areas towards the flower end of the fruit where the sun has bleached the skin to a yellow to white colour.
- No single banana on an outside whorl of a hand or cluster, (except a wing banana or distorted banana) has a diameter that exceeds 42 mm when measured at right angles to the curvature of the fruit, at a point one third from its flower end.
- The skin has no pre-harvest cracking, punctures, pulled stem or other breaks that penetrate through to the flesh and has not healed with callus tissue.

Inspection records for hard green Philippine bananas imported into New Zealand, Japan and Korea show that no *B. philippinensis* or *B. occipitalis* fruit flies have been intercepted on hard green bananas to date (Iwaizumi 2004; Biosecurity New Zealand MAF, Wellington, New Zealand 2005a; F Kang, Senior Product Management Manager, Del Monte Fresh Produce Korea Ltd, pers comm 16 February 2006; Biosecurity New Zealand MAF, Wellington, New Zealand, 11 January 2006).

Appendix 11. Armoured scales

The Diaspididae, commonly armoured scales, is the largest family in the superfamily Coccoidea and members of the order Hemiptera. A wide range of economic plants are subject to attack by these insects and several species of armoured scales are of economic importance, as they are destructive pests (Ben-Dov and German 2002).

Diaspidids usually feed on perennial host plants, mainly tree species. The species they attack include almond, apple, avocado, banana, cassava, citrus, cocoa, coconut, coffee, durian, fig, ginger, grape, guava, mango, oil palm, papaya, pecan, pineapple, rambutan, rubber, sugarcane, tea, turmeric, walnut and yam as well as various forest trees and ornamentals (Chua and Wood 1990). Although diaspidids attack plants, only about 10% of species are serious pests, probably because they are normally controlled by natural enemies.

At least 21 species of diaspidids have been recorded on banana plants worldwide. Bananas covered in scale may be unacceptable for sale and especially for export. Severe outbreaks have only been reported in Israel where entire crops have been lost, probably because of the lower numbers of parasitoids (Chua and Wood 1990).

The following three diaspidid scales are of quarantine significance to the whole of Australia and occur on bananas in the Philippines:

11.1 *Aspidiotus coryphae* (Cockerell and Robinson 1915)
11.2 *Aspidiotus excisus* (Green 1896)
11.3 *Pinnaspis musae* (Takagi 1963).

The following species are of quarantine significance to Western Australia:

11.4 *Abgrallaspis cyanophylli* (Signoret 1869)
11.5 *Hemiberlesia palmae* (Cockerell 1892)
11.6 *Pseudaulacaspis cockerelli* (Codey 1897)
11.7 *Selenaspidus articulatus* (Morgan 1889).

A general description of the biology of Diaspididae follows. The information is mainly taken from Williams and Watson (1988), Rosen (1990) and Schaefer and Panizzi (2000) and from information provided by the Philippine Government (BPI 2001).

Adult female Diaspididae produce a fibrous scale (armour) that incorporates the cast off exoskeletons (exuviae) of earlier instars. Eggs are laid under the armour of the female, where they hatch and develop. Hatching takes from several hours to several days. Females produce from one to 10 eggs a day and continue laying for several weeks until their death (Koteja 1990a).

The first stage after hatching is called a crawler. Females only have two nymphal stages and crawlers are the only nymphal stage of females with legs. The crawlers may stay under the maternal armour from several minutes to several hours in a torpid state until external conditions such as temperature and humidity are favourable. Once these conditions are met and they emerge from under the female, they are mobile and wander for a period ranging from minutes to days, but on average lasting a few hours, searching for a suitable feeding site.

Commonly, crawlers are most active between 25–32 °C, with 13 °C being the threshold temperature for activity. Light intensity must also be above 32 lux for crawlers to emerge. Dark periods synchronise emergence and maximum emergence in natural conditions is during the early morning. They are able to walk up to 150 m, but usually settle within 50 cm or so of the maternal female, and usually within 24 hours of emerging. They may wander more on yellow fruits, but less on green fruits (Greathead 1990). Each crawler walks to an exposed position on the plant, from which air currents

may carry it up to several kilometres away. According to Greathead (1990), infestations have been recorded at distances as great as 80 km downwind of a source.

Movement of infested planting material or produce is the main way by which diaspidids have been introduced to other countries. The most common mode of dispersal of sessile stages is on plant parts transported by human activities. In particular, long-range dispersal of the sessile female scale can only occur by means of passive transport on infested plant material. Passing animals or people can also can carry crawlers over great distances. The crawler stage can be carried by other vertebrates such as birds and invertebrates, particularly ants, as well as wind currents. Although wind is an agent of dispersal, it can also cause mortality because crawlers dislodged by wind may not land on a host plant (Koteja 1990b). Crawlers suffer high mortality (G Watson, Associate Insect Biosystematist, California Department of Food and Agriculture, pers comm 18 February 2006).

At the end of the wandering period, they secure themselves on a leaf or stem with their mouthparts. Crawlers do not feed until they settle and prefer a rough or dusty surface of a young leaf to settle on. Once settled, the larvae draw their legs beneath the body and flatten themselves against the host. They then insert their piercing and sucking mouthparts into the plant tissue and start feeding on plant juices. The tips of their mouthparts are in the form of a tubular stylet. A large dorsal canal in the stylet functions as a liquid food canal, while a smaller ventral canal transmits the digestive enzymes.

The stylets penetrate tissues either between epidermal cells or through stomata. All cells penetrated during insertion are killed until the stylets reach a feeding site in the phloem. Once inserted, the stylets remain in place throughout the life of the scale.

Scales continue to grow and moult until they reach a certain size. After growth has started, a change of feeding site seems impossible in this group (Koteja 1990b). Male and female crawlers usually cannot be distinguished morphologically, but may exhibit different behaviours. The females only pass through two growth stages and then become sessile. The armour is secreted once the nymphs became sessile and the exoskeleton is shed twice as they grow and develop. The cast exoskeletons are incorporated into the armour. The armour is made of cast skins, threads and liquid secreted by the insect. Females remain under the armour throughout their lives feeding, growing and reproducing, while adult males are winged.

Males, when present, have five instars with four immature stages: first-instar nymph, second-instar nymph, pre-pupa, pupa and then adult. The male's scale covering is always smaller than that of the female, and the second instar is the last feeding stage. Subsequently, the male passes through two pupal instars before becoming adult. The adult male emerges as a delicate, gnat-like insect that lives from a few hours to a maximum of three days. It has no functioning mouthparts and normally has a single pair of wings, although some are wingless. It also has well developed legs and antennae. Apart from the crawlers, the adult male is the only mobile stage (Koteja 1990b). There is often only one generation of males produced each year.

Climatic factors, in particular temperature and humidity, influence every aspect of the scale life history. Survival and fecundity are also positively influenced by high foliar nitrogen. Some species are able to undergo winter diapause and summer diapause has been noted in a small number of species (McClure 1990a).

In many sexually reproducing species of scales, the ratio of males to females is close to 1:2 in larvae and later stages, although more commonly it is considerably less. This may be due to the lower survival rate of immature males compared to females in some species (Nur 1990a). The sex ratio can also be influenced by external factors and parasitism. It can also be altered by the age of the female and the time elapsed since mating. Increases in these factors result in an increase in the number of males produced. The effect of temperature is similar: if females raised at around 25 °C are exposed to temperatures of 15 °C for a few hours shortly after mating, they produce more males (Nur 1990a). Also, if mating is delayed, they produce more males than females.

It is thought that this is an adaptive shift. The normal pattern is that females tend to be produced earlier by the maternal scale than males, as females require more and better quality food than males (Nur 1990a).

Parthenogenesis is fairly common among scale species, particularly in the worst pest species (Nur 1990b), but sexual reproduction as well as parthenogenesis can occur in a single species. Sometimes parthenogenesis is initiated by a pathogen (Provencher et al 2005).

Armoured scales affect their hosts by removing sap, as well as by injecting toxic saliva during feeding. The feeding process results in cell death, deformation of plant parts and the formation of galls and pits, as well as increased susceptibility to other destructive agents such as frost, disease and other pests (McClure 1990a). High populations of scales can cause the death of trees (Beardsley and Gonzalez 1975; Smith et al 1997).

Some armoured scales have demonstrated an ability to greatly expand their host range when exposed to a new environment. Their range may therefore be due to past and current exposure to possible hosts rather than reflecting the limits of adaptation to the attributes of known hosts (McClure 1990b).

Scale insects have both a direct and an indirect relationship with ants. It has been shown that the presence of ants reduces the effectiveness of the scale's natural enemies. However, unlike some other insects, diaspidid scales do not produce honeydew and therefore direct relationships with ants are less common. Recently, a direct association has been discovered between a scaleless diaspidid and a small genus of ants in Africa whereby the scale lives in the galleries of the ant nest and is tended by the ants, but this is exceptional (Ben-Dov 1990).

Numerous predators of scale insects are known. They include coccinellid beetles, other beetles such as Staphylinidae, some Nitidulidae, Anthribidae, Cantharidae and Tenebrionidae; Neuroptera (lacewings); Cecidomyidae and other predatory flies; some Thysanoptera (thrips); and certain Acari (mites). Parasitoids (mainly Hymenoptera) are the most important natural enemies of armoured scale. Natural enemies regulate the size of the armoured scale population. However, they do not operate independently, but rather in conjunction with other factors such as climate and the genetic makeup of the population. Disease may also play a role in regulating population densities of armoured scale insects.

11.1 *Aspidiotus coryphae*

Scientific name

Aspidiotus coryphae Cockerell and Robinson [Hemiptera: Diaspididae]

Synonyms

None

Common name

Corypha scale

Hosts

Aspidiotus coryphae has been reported on *Cocos nucifera, Corypha elata* (Munting 1971) and *Cycas revoluta* (Watson 2005), and has been intercepted on Philippine bananas exported to Japan (Sugimuto 1984).

Cocos nucifera (coconut palm) (Munting 1971); *Corypha elata* (Gebang palm) (Munting 1971); *Cycas revolute* (sago palm) (Watson 2005); *Musa* spp. (banana) (Sugimoto 1984).

Plant parts affected

Aspidiotus destructor affects leaves, stems, growing points and fruits (Tabibullah and Gabriel 1973), and *A. coryphae* would have similar affect.

Distribution

The species has only been found in the Philippines (Sugimoto 1984; Spence 2002)

Biology

No specific published information on the biology of this particular species is available. However, the biology of *A. coryphae* is similar to that of *A. destructor* because they are in the same genus.

The lifecycle of *A. destructor* typically lasts for 32–34 days. In one study, the lifecycle was found to be 32 days for females and 27 days for males. The larvae and the adult males are the only mobile stages (Tabibullah and Gabriel 1973).

The eggs of *A. destructor* are laid under the scale of the adult female. The female deposits 20–50 eggs under her scale over a few days. The eggs are incubated for 7–8 days. After hatching, the nymphs crawl out from under the scale edge and colonise the undersurface of the leaf.

The females have two immature stages. The males have four immature stages: first instar nymph, second instar nymph, pre-pupa and pupa.

The first-instar nymph (crawler) leaves the maternal scale and begins feeding on the leaves of the host. It is mobile in both sexes. Crawlers are found on the undersides of leaves and tender shoots and on leaf tips. They drop off the leaves easily and may be dispersed by the wind. Damage from these scales is reduced during the rainy season.

Individual females in each generation lay an average of 32–42 eggs. At room temperature (26–28 °C), the egg stage lasts for five days, the nymphal stage lasts 17 days, the pre-oviposition stage in adult females lasts 25 days, the female generation lasts 44 days and the male generation lasts 38 days.

11.2 *Aspidiotus excisus*

Scientific name

Aspidiotus excisus (Green 1896) [Hemiptera: Diaspididae]

Synonyms

Aspidiotus (Aspidiotus) excisus (Cockerell 1897)
Aspidiotus (Evaspidiotus) excisus (Leonardi 1898c)
Temnaspidiotus excisus (MacGillivray 1921)

Common names

Cyanotis scale (Velasquez and Rimando 1969); Aglaonema scale (Miller and Davidson 2005).

Hosts

Aspidiotus excisus is considered a pest of ornamental plants (Dekle 1976; Davidson and Miller 1990). It has been recorded from 25 families, but most intercepted material in collections from quarantine is from *Aglaonema*, *Citrus* and *Lonicera* (Miller and Davidson 2005).

Adenium sp. (desert rose) (Miller and Davidson 2005); *Aechmea* sp. (bromeliad) (Miller and Davidson 2005); *Aglaonema commutatum* (Chinese evergreen) (Velasquez 1971); *Alocasia* sp. (Miller and Davidson 2005); *Carica papaya* (papaya) (Takagi 1969; Williams and Watson 1988); *Caryota* sp. (fishtail palms); *Chlorophytum* sp. (Miller and Davidson 2005); *Citrus* sp. (Takagi 1969; Williams and Watson 1988); *Citrus aurantifolia* (key lime) (Williams and Watson 1988); *Clerodendron inerme* (scrambling Clerodendron) (Takahashi 1929, 1936; Green 1937; Ferris 1941); *Clerodendrum neriifolium* (Takagi 1969); *Cocos nucifera* (coconut palm) (Takahashi 1941; Takagi 1969); *Coffea* sp.; *Cyanotis* sp. (Green 1937); *Cyanotis pilosa* (Green 1896; Ramakrishna 1921; Takagi 1969; Tang 1984); *Cycas* sp. (Miller and Davidson 2005); *Elephantopus mollis* (Takahashi 1929; Takagi 1969; Tang 1984); *Euphorbia* sp. (Williams and Watson 1988); *Gardenia* sp.; *Glochidion hongkongense* (Hong Kong abacus plant) (Takagi 1969); *Hoya carnosa* (Takahashi 1932, 1933; Takagi 1969); *Ipomoea* sp. (Green 1900, 1937; Takagi 1969; Tang 1984); *Jacobinia* sp. (Miller and Davidson 2005); *Lonicera* sp. (Miller and Davidson 2005); *Mangifera* sp. (Miller and Davidson 2005); *Melicoccus* sp. (Miller and Davidson 2005); *Murraya* sp., (Miller and Davidson 2005); *Musa* sp. (Takagi 1969); *Pentas* sp. (Miller and Davidson 2005); *Piper* sp. (Green 1937; Takagi 1969; Tang 1984); *Pritchardia* sp.; *Psidium* sp. (Miller and Davidson 2005; Velasquez 1971); *Rhododendron* sp. (Takahashi 1936; Takagi 1969); *Rhus semialata* (Takahashi 1932, 1933; Takagi 1969); *Thespesia* sp. (Beardsley 1966; Takagi 1969); *Thespesia populnea* (Portia tree) (Beardsley 1966); *Tournefortia* sp. (Beardsley 1966; Takagi 1969; Tang 1984); *Tournefortia argentea* (Takahashi 1942); *Urena lobata* (Takahashi 1932, 1933; Takagi 1969); *Viburnum* sp. (Takahashi 1932, 1933; Takagi 1969).

Plant parts affected

Aspidotus excisus normally lives on leaves and green stems, but it has been detected on banana fruit from the Philippines entering Japan and New Zealand. It therefore appears to feed on fruit as well as other parts of the banana plant, based on the interception data (Sugimoto 1994).

Distribution

Table 11.1 lists the countries where *A. excisus* has been recorded.

Table 11.1 Distribution list for *Aspidotus excisus*

Country	References	Country	References
Antigua and Barbuda – Antigua	Nakahara (1982)	Mongolia	Danzig and Konstantinova (1990)
China	Tang (1984)	Pakistan	Nakahara (1982)
Colombia	Kondo (2001)	Palau	Takahashi (1942); Beardsley (1966)
Costa Rica	Nakahara (1982)	Panama	Nakahara (1982)
Dominican Republic	Nakahara (1982)	Papua New Guinea	Williams and Watson (1988)
Ecuador	Nakahara (1982)	Philippines	Velasquez and Rimando (1969); Velasquez (1971); Stephens (1984)
El Salvador	Nakahara (1982)	Puerto Rico (Puerto Rico and Vieques Island)	Col-Ferrer and Medina-Gaud (1998)
Federated States of Micronesia – Caroline Islands	Takahashi (1941); Beardsley (1966)	Saint Croix	Nakahara (1982)
Fiji	Green (1937)	Samar	Velasquez (1971)
Grenada	Nakahara (1982)	Singapore	Nakahara (1982)
Guatemala	Nakahara (1982)	Sri Lanka	Green (1896, 1900, 1937); Ramakrishna (1921); Takagi (1969)
Guyana	Nakahara (1982)	Surinam	Nakahara (1982)
Honduras	Nakahara (1982)	Taiwan	Takahashi (1929, 1932, 1936); Green (1937); Takagi (1969); Wong et al (1999)
India	Nakahara (1982)	Thailand	Takahashi (1942a); Takagi (1969)
Indonesia	Nakahara (1982)	Trinidad and Tobago – Trinidad	Nakahara (1982)
Jamaica	Nakahara (1982)	Tripura	Varshney (2002)
Japan	Kawai (1980)	United States of America – Florida	Dekle (1965)
Martinique	Nakahara (1982)	US Virgin Islands	Nakahara (1983)
Mexico	Nakahara (1982)	Venezuela	Nakahara (1982)

Biology

Female scales are approximately circular, convex, of irregular border with a margin that is often lobed, and a body that is thin and semi-transparent, whitish, fawn or very pale ochre, and visible through the scale cover. The exuviae are slightly yellow and approximately central. The male is smaller and more elongate, with exuviae at one end, the same colour as for the female. The female is about 0.75 cm long when slide mounted (Ferris 1941; Velasquez 1971; Tang 1984; Chou 1985, 1986; Williams and Watson 1988). Eggs are yellow.

Aspidiotus excisus is commonly encountered on the leaves of its host, where it may build up heavy populations. Takahashi (1936) reported that this species caused gall-like folds on the leaves of *Clerodendron inerme* (Miller and Davidson 2005). Dekle (1976) assessed the species as being a serious pest in Florida on *Aglaonema* spp. and *Hoya carnosa*. Jepson (1915) noted that the species was a serious pest on bananas in Fiji. Talhouk (1975) recorded that it was of economic importance to some extent in India and China on citrus. Miller and Davidson (2005) considered that this species could be a serious pest in some parts of the world.

Coccoids are believed to have been intercepted on exports from the Netherlands and Japan to the United States. Species mentioned include *A. excisus* (Fox-Wilson 1939). Sugimoto (1994) recorded *A. excisus* in Japanese quarantine inspections of bananas imported from the Philippines. It has been intercepted on numerous occasions by New Zealand quarantine authorities on bananas imported from the Philippines (Biosecurity New Zealand MAF, Wellington, New Zealand 2005a).

11.3 *Pinnaspis musae*

Scientific name

Pinnaspis musae Takagi 1963 [Hemiptera: Diaspididae]

Synonyms

None

Common names

None

Hosts

Musa spp. (banana) (Takagi 1963; Sugimoto 1994). No other published information could be obtained for this species. However, some other species of *Pinnaspis*, such as *P. strachani* (Cooley) are polyphagous.

Plant parts affected

The cogeneric species *P. strachani* is found on fruits, leaves, and stems of its host plants (CAB International 2001). *P. musae* may be similar.

Distribution

The species has only been found in the Philippines (Sugimoto 1994).

Biology

No specific published information on the biology of *P. musae* is available. The biology of a cogeneric species *P. buxi* (Bouche) on bananas in Hawaii prepared by Tenbrink (1992a) is presented below.

Eggs are laid under the armour of the female where they develop and hatch. The first stage after hatching is the only nymphal stage with legs, so the insects are called crawlers. Crawlers may stay under the maternal armour for several hours until outside conditions, especially temperature and humidity, are good. After they leave the maternal cover, they wander for a period ranging from minutes to days, but usually a few hours. At the end of the wandering period they flatten against the leaf or stem and begin to secrete their armour. Newly settled nymphs insert their piercing, sucking mouthparts into plant tissue and start feeding on plant juices. Nymphs shed their exoskeletons twice as they grow and develop. The cast exoskeletons, called exuviae, are incorporated into the armour at the narrow end, forming a dot. The armour is non-living and is made of cast skins, threads and liquid, all produced by the insect. Females remain under the armour in one place throughout their lives to feed and reproduce.

Since female armoured scales are not capable of wandering after they have settled and started feeding, long-range dispersal happens by passive transport of infested plant material. Short-range dispersal happens as crawlers search out places to settle and feed. It is the crawler stage that can be carried directly from place to place by people, animals, birds, ants and wind currents. Wind is an agent of dispersal and also one of mortality, since crawlers dislodged by wind may not land on suitable host plants.

11.4 *Abgrallaspis cyanophylli*

Scientific name

Abgrallaspis cyanophylli (Signoret 1869) [Hemiptera: Diaspididae]

Synonyms

Abgrallaspis cyanophylli: Aspidiotus cyanophylli (Signoret 1869*)*
Fucaspis cyanophylli (Signoret)
Hemiberlesia cyanophylli (Signoret)

Common name

Cyanophyllum scale

Hosts

From Williams and Watson 1988; and CAB International (2006):

Abgrallaspis cyanophylli; Acalypha hispida (chenille plant); *Annona* sp. (custard apple); *Annona squamosa* (sugar apple); *Artocarpus altilis* (breadfruit); *Bauhinia* sp.; *Barringtonia* sp.; *Camellia sinensis* (tea); *Capsicum ovatum; Ceiba pentandra* (kapok tree); *Cinnamomum verum* (cinnamon*); Clerodendrum* sp.; *Coccoloba uvifera* (Jamaican kino, sea-grape); *Cocos nucifera* (coconut*); Coffea arabica* (arabica coffee); *Coffea* sp. (coffee); *Coleus* sp.; *Cordyline fruticosa* (palm lily); *Dioscorea alata* (greater yam); *Dioscorea* sp. (yam); *Elettaria cardamomum* (cardamom); *Eriobotrya japonica* (loquat); *Eugenia* sp.; *Ficus* sp. (fig); *Guettarda speciosa* (beach gardenia); *Hevea brasiliensis* (rubber tree); *Hibiscus syriacus (*rose-of-Sharon*); Jatropha curcas* (Barbados-nut, physic nut); *Macadamia tetraphylla (*rough-shell Queensland nut); *Mangifera indica* (mango); *Manihot esculenta* (cassava, tapioca); *Musa* × *paradisiaca* (banana); *Musa* sp. (banana); *Persea americana* (avocado); *Piper methysticum* (kava kava); *Plumeria rubra* f. *acutifolia* (Mexican frangipani, pagoda tree); *Psidium guajava* (guava); *Nerium* sp. (oleander, rose laurel); *Swietenia macrophylla* (Honduras mahogany); *Theobroma cacao* (cocoa); *Toona ciliata* (Australian red cedar).

Plant parts affected

The plant parts affected include leaves, stems and fruit (Watson 2008).

Distribution

Table 11.2 lists the countries where *A. cyanophylli* has been recorded.

Table 11.2 Distribution list for Abgrallaspis cyanophylli

Country	Reference	Country	Reference
Australia (New South Wales, Queensland and Tasmania, but not in Western Australia)	CSIRO-AFFA (2004a)	Kiribati	Williams and Watson (1988)
		New Caledonia	Williams and Watson (1988)
		Papua New Guinea	Williams and Watson (1988)
Cook Islands	Williams and Watson (1988)	Philippines	Sagimoto (1994)
Fiji	Williams and Watson (1988)	Tonga	Williams and Watson (1988)
French Polynesia (Tahiti)	Williams and Watson (1988)	Tuvalu	Williams and Watson (1988)
Georgia	Watson (2008)	Vanuatu	Williams and Watson (1988)
India (Tamil Nadu)	Watson (2008)	Western Samoa	Williams and Watson (1988)

Biology

There are several generations of *A. cyanophylli* each year in California (Gill 1997), and up to five per year in northern Taiwan (Shiao 1979). In Taiwan, climatic factors were found to account for 35.5% of mortality of *A. cyanophylli* (Hsiao 1981). He et al (1998), found that it reproduced most successfully at 28 °C and 75% relative humidity; they found that the nymphal stages all together lasted 37–64.5 days at 20 to 28 °C. Crawlers are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Mortality of crawlers due to abiotic factors is high. Dispersal of sessile adults and eggs occurs through human transport of infested plant material.

11.5 *Hemiberlesia palmae*

Scientific name

Hemiberlesia palmae [Hemiptera: Diaspididae]

Synonyms

Aspidiotus palmae
Aspidiotus unguiculatus
Aspidiotus javanensis
Abgrallaspis palmae
Borchseniaspis palmae

Common names

Dark-spotted scale; tropical palm scale; coconut scale.

Hosts

Hemiberlesia palmae is highly polyphagous and feeds on more than 110 reported wild and commercial host plants in over 50 plant families (Ben-Dov et al 2006c). *H. palmae* is a pest of crops in the tropics, such as banana, coconut palm, oil palm, manihot, tea and cocoa (Chua and Wood 1990; Watson 2005) occasionally damaging orchids and palms in greenhouses in cooler regions.

From (Chua and Wood 1990 and Watson 2005):

Camellia sinensis (tea); *Cocos nucifera* (coconut palm); *Elaeis* sp. (oil palm); *Manihot esculenta* (manihot); *Musa* sp. (banana); *Theobroma cacao* (cocoa).

Plant parts affected

The plant parts affected include leaves and, to a lesser extent, fruit (Watson 2005; Ben-Dov et al 2006c). It is found in high numbers on the leaves of its hosts, especially on palms.

Distribution

This scale is widely distributed throughout the world and is found in temperate and tropical countries on every continent.

Biology

The biology of this scale insect has apparently not been studied. Crawlers are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Mortality due to abiotic factors is high in this stage. Dispersal of sessile adults and eggs occurs through human transport of infested plant material (Watson 2005).

11.6 *Pseudaulacaspis cockerelli*

Scientific name

Pseudaulacaspis cockerelli [Hemiptera: Diaspididae]

Synonyms

Chionaspis dilatata
Phenacaspis natalensis
Phenacaspis aucubae
Phenacaspis cockerelli
Phenacaspis dilatata

Common names

Cockerell scale; false oleander scale; Fullaway oleander scale; magnolia white scale; mango scale; oleander scale; oyster scale.

Hosts

Pseudaulacaspis cockerelli is a highly polyphagous species that has been recorded from 194 wild and commercial host species belonging to 68 plant families (Ben-Dov et al 2006d). This species is particularly common on ornamental plants (Watson 2005), coconut (Williams and Watson 1988), other palms and magnolias. Host plants include *Aucuba* sp.; *Bischofia* sp.; *Camellia* sp.; *Campnosperma* sp.; *Carica papaya* (papaya); *Chrysalidocarpus* sp.; *Cocos nucifera* (coconut palm); *Euonymus* sp.; *Eurya* sp.; *Excoecaria* sp.; *Hedera* sp.; *Liquidamber* sp.; *Magnolia grandiflora*; *Mangifera indica* (mango); *Michelia* sp.; *Nerium oleander* (oleander); *Nypa fruticans* (Nipa palm); *Palmae* sp.; *Plumeria* sp.; *Rhododendron* sp.; *Ribes* sp.; *Syringa* sp.; *Taxus* sp.; *Trochodendron* sp. and *Viburnum* sp. (Tenbrink and Hara 1992b; Watson 2005).

Plant parts affected

Leaves, green stems and occasionally twigs (Tenbrink and Hara 1992b; Watson 2005).

Distribution

Table 11.3 lists those countries where *P. cockerelli* has been recorded.

Table 11.3 Distribution list for Pseudaulacaspis cockerelli

Country	References
Africa (eastern and southern)	Watson (2008)
Asia	Watson (2008)
Australia	Watson (2005)
Central America	Watson (2008)
Pacific region	Watson (2005)
Philippines	Watson (2008); Spence (2002)
USA	Watson (2005)

Biology

Females lay tiny yellow eggs under their armour. Eggs can be in large groups (more than 20). The first stage after hatching is the only nymphal stage with legs, so the insects are called crawlers. Crawlers

may stay under the maternal armour several hours until outside conditions, especially temperature and humidity, are good. After they leave the maternal cover they wander for a period ranging from minutes to days, but usually lasting a few hours. At the end of the wandering period they flatten against the leaf or stem and begin to secrete their armour.

Newly settled nymphs insert their piercing, sucking mouthparts into plant tissue and start feeding on plant juices. Nymphs shed their skin as they grow and develop (twice in females and four times in males). The cast skin, called exuviae, is incorporated into the armour at the narrow end forming a yellowish brown spot in the otherwise white armour. Males feed only during the first and second stages and do not enlarge their armour after that. The armour of males and females is non-living and is made of cast skins, threads and liquid, all produced by the insect (Beardsley and Gonzalez 1975).

As the female matures, she becomes an oval, bright yellow, feeding and egg-laying body hidden under white pear-shaped armour. Males are protected by armour until they develop into tiny winged adults that are very different in appearance and behaviour from females.

The number of days for each developmental stage and the number of generations per year depend on temperature, humidity and rainfall (Beardsley and Gonzalez 1975). Based on a generalised life history of other tropical species, 30 days is the approximate time to complete the lifecycle from egg to reproducing adult (Tenbrink and Hara 1992b).

Crawlers are the primary dispersal stage and move to new areas of the plant or are dispersed by wind or animal contact. Mortality due to abiotic factors is high in this stage. Dispersal of sessile adults and eggs occurs through human transport of infested plant material (Watson 2005).

11.7 *Selenaspidus articulatus*

Scientific name

Selenaspidus articulatus [Hemiptera: Diaspididae]

Synonyms

Aspidiotus articulatus
Selenaspidus articulatus simplex

Common names

Rufous scale; West Indian red scale.

Hosts

Selenaspidus articulatus is a highly polyphagous species that has been recorded from 122 wild and commercial host species belonging to 48 plant families (Ben-Dov et al 2008g). This species is an important pest of commercial plants, particularly citrus, coffee and olives (Watson 2008). Other host plants include *Annona squamosa* (custard apple); *Averrhoa carambola* (Carambola); *Camellia sinesnsis* (tea); *Carica papaya* (papaya); *Cocos nucifera* (coconut palm); *Mangifera indica* (mango); *Musa sapientum* (banana); *Passiflora edulis* (passionfruit); *Persea americana* (avocado); and *Vitis vinifera* (grapes) (Ben-Dov et al 2008g).

Plant parts affected

Leaves and sometimes fruits/pods, growing points and stems (Watson 2008).

Distribution

This scale is widely distributed throughout the world and is found in temperate and tropical countries on every continent.

Biology

The biology of *S. articulatus* is summarised by Watson (2008). The lifecycle (from egg to adult) takes 30 days for the male and 45 days for the female. Females commence reproduction at day 45, peaking at day 80. On citrus, each female typically produces between 71 and 124 eggs. *Selenaspidus articulatus* is an ovoviviparous species and emergence of the crawlers takes place immediately after the egg is laid. Crawlers are subject to high mortality and are the primary dispersal stage, moving to new areas of the plant or are dispersed by wind or animal contact.

Females typically develop through the following stages: crawler; first; second and third sessile instars; immature adult (pigidium protected); and mature female (pigidium retracted). The third instar is delayed in males and the pupa is recognizable by the oval shape of the scale cover and the scutiform shape of the body.

Moraes et al (2000) estimated the optimal temperature for the development of male and female *S. articulatus* to be 28 ºC and the upper threshold temperatures to be 30.3 and 30.2 ºC respectively. Loayza et al (2003) studied the biology of *S. articulatus* reared on four *Citrus sinensis* varieties and found that the rate of development for both males and females varied significantly with variety. At 25 ± 2 ºC and 60 ± 10% RH, female development varied from 25.4 – 42.1 days and males 24.4 – 39.9 days. Mortality was also found to vary for both males and females, particularly among immature instars of both sexes.

Appendix 12. Mealybugs

The following mealybugs are of quarantine significance to the whole of Australia and occur on bananas in the Philippines:

12.1 *Dysmicoccus neobrevipes* Beardsley 1959 [Hemiptera; Pseudococcidae]
12.2 *Nipaecocccus nipae* Maskell 1893 [Hemiptera; Pseudococcidae]
12.3 *Pseudococcus jackbeardsleyi* Gimpel and Miller 1996 [Hemiptera; Pseudococcidae]

The following species is of quarantine significance to Western Australia:

12.4 *Planococcus minor* (Maskell 1897) [Hemiptera; Pseudococcidae]

Information about these four species is provided on the following pages.

12.1 *Dysmicoccus neobrevipes*

Scientific name

Dysmicoccus neobrevipes Beardsley 1959 [Hemiptera: Pseudococcidae]

Prior to 1959, *D. neobrevipes* was considered the 'grey' form of the pineapple mealybug *Dysmicoccus brevipes* (Cockerell) species complex. In Hawaii, observed biological differences between the pink and grey 'strains' of pineapple mealybug led to the discovery of characteristics that enabled taxonomic separation, and the grey bisexual form was recognised as a distinct species, *D. neobrevipes* Beardsley (Beardsley 1993). *D. neobrevipes* Beardsley is very close to *D. brevipes* (Williams 2004), which is found in Australia.

Common names

Annona mealybug, grey/gray pineapple mealybug, pineapple grey mealybug.

Hosts

Dysmicoccus neobrevipes is polyphagous and has been recorded on 84 hosts from 45 plant families. These hosts include commercial fruit, vegetable and tree crops, non-commercial plants, ornamental plants and a number of weeds. The following host list was extracted from Beardsley (1959), Williams and Watson (1988), Ben-Dov (1994), Ben-Dov et al (2005a) and Williams (2004).

Acacia farnesiana (huisache); *Acacia koa; Agave sisalana* (sisal); *Aglaonema treubii; Albizia saman* (rain tree); *Allium cepa* (onion); *Alpinia purpurata* (red ginger); *Ananas comosus* (pineapple); *Ananas sativus; Annona muricatae* (sour sop); *Annona reticulata* (custard apple); *Annona squamosa* (sweet sop); *Arachis hypogaea* (peanut); *Artocarpus altilis* (bread fruit); *Artocarpus heterophyllus* (jackfruit); *Barringtonia speciosa; Brassavola cordata* (orchid species); *Brassica oleracea* (cabbage); *Cajanus cajan* (pigeon pea); *Chrysalidocarpus lutescens* (yellow butterfly palm); *Citrus aurantifolia; Citrus limon* (lemon); *Citrus sinensis* (sweet orange); *Clerodendrum* sp.; *Coccoloba* sp.; *Coccoloba uvifera* (sea grape); *Cocos nucifera* (coconut); *Codiaeum* sp.; *Coffea arabica* (coffee); *Coffea canephora* (robusta coffee); *Colocasia esculenta* (taro); *Cordia alliodora* (laurel); *Crescentia alata* (calabash tree); *Cucurbita maxima* (squash); *Ficus* sp. (fig); *Garcinia mangostana* (mangosteen); *Gossypium* (cotton); *Guettarda speciosa; Helianthus annuus* (sunflower); *Heliconia latispatha* ('Golden Torch' heliconia); *Impatiens* sp. (Busy Lizzy*); Lansium domesticum* (langsat); *Lycopersicon esculentum* (tomato); *Machaerium robinifolium; Mangifera indica* (mango*); Manilkara zapota* (sapodilla); *Messerschmidia argentea; Millingtonia hortensis* (tree jasmine); *Musa* sp.; *Musa coccinea* (scarlet banana); *Musa paradisiaca* (banana); *Mussaenda oona; Nephelium lappaceum* (rambutan); *Nothopanax* sp.; *Opuntia megacantha* (cactus); *Pandanus* sp.; *Phaseolus* (bean); *Philodendron* sp.; *Pinus* sp (pine); *Pipturus argentea; Piscidia piscipula; Plumeria acuminata* (frangipani); *Polianthes tuberosa* (tuberose); *Psidium guajava* (guava); *Punica granatum* (pomegranate); *Samanea saman* (saman); *Solanum melongena* (eggplant); *Spathodea campanulata* (African tulip tree); *Syzygium malaccensis* (mountain apple, jambu); *Tamarindus indica* (tamarind); *Tamarix articulate* (Athel pine); *Tectona grandis* (teak); *Theobroma cacao* (cocoa); *Thespesia propulnea; Tournefortia argentea; Vigna sesquipedalis* (asparagus bean); *Yucca elephantipes; Zea mays* (maize).

Plant parts affected

Developing fruit (pineapple) (Beardsley 1993).

Aerial parts of host plant – that is, flower, fruit clusters, leaves, stem and aerial roots (Kessing and Mau 1992).

Banana fruit and pseudostem (PCARRD 1988).

Distribution

Dysmicoccus neobrevipes has expanded its geographical range extensively since 1945, but is still unknown in pineapple-growing areas of Africa, Asia and Australia (Beardsley 1993). Table 12.1 lists the countries where *D. neobrevipes* has been recorded.

Table 12.1 Distribution list for Dysmicoccus neobrevipes

Source: Williams (2004) and Ben-Dov et al (2005a)

American Samoa	Haiti	Panama
Antigua and Barbuda	Hawaiian Islands	Peru
Bahamas	Honduras	Philippines
Brazil	India	Puerto Rico (Puerto Rico and Vieques Island)
Colombia	Italy (Sicily)	Singapore
Cook Islands	Jamaica	Surinam
Costa Rica	Kiribati	Trinidad and Tobago (Trinidad)
Dominican Republic	Malaysia (Sabah)	United States of America
Ecuador	Marshall Islands	US Virgin Islands
El Salvador	Mexico	Vietnam
Fiji	Northern Mariana Islands	Western Samoa
Guam	Pakistan	
Guatemala		

Note: BPI (2001) lists *D. neobrevipes* as occurring in Australia and cites Woodward et al (1970) as evidence. However, *D. neobrevipes* is actually not listed anywhere in the book, but *D. brevipes* (Cockerell) is listed as a pest of pineapple on page 430 of Woodward et al (1970).

In Hawaii, both *D. brevipes* and *D. neobrevipes* are reported to transmit pineapple wilt disease, but only *D. neobrevipes* causes green spot on pineapple (Beardsley 1965). Because green spotting was reported by Carter (1942) in Queensland, presumably, this is the basis on which BPI (2001) regards some records of *D. brevipes* in Australia as being *D. neobrevipe*s. In its submission to the draft IRA report, the Philippines Government argues that 'where Williams (1985), based on available specimens, reported only *D. brevipes* in his book on Australian mealybugs, the remarks under that species hint at a possible occurrence of *D. neobrevipes* in Australia'. This is not totally correct. Williams' (1985: p.118) exact statement reads; 'No specimens [of *D. neobrevipes*] have been found so far in Australia, despite the record by Carter (1942) that green spotting occurs on pineapples in Queensland'. Williams (1985) then tried to explain why this may be the case using Beardsley's (1965) work, as follows:

There are two forms of D. brevipes: one is a parthenogenetic form that transmits only pineapple wilt in Hawaii; the other is a bisexual (biparental) form that apparently causes green spotting in Brazil. Although all the specimens examined by Williams (1985) were female, the record of green spotting in Queensland led him to suggest that the bisexual form may be present there. It is clear that Williams' (1985) explanation of the record of the presence of green spotting on pineapple in Queensland is due to the possible presence of the bisexual form of D. brevipes, not D. neobrevipes. This is supported by the fact that he examined numerous specimens of D. brevipes from Queensland, New South Wales, Western Australia and the Northern Territory and found no specimens of D. neobrevipes. In addition, Ben-Dov and German (2002) do not include Australia in the known distribution of D. neobrevipes.

These two species coexist in many instances in some countries, but D. brevipes appears to be much more widespread – see lists of distribution for these two species in Ben-Dov and German (2002) – and many countries still do not have records of D. neobrevipes, although D. brevipes has been reported there. Therefore, there is no evidence to support the contention that D. neobrevipes is present in Australia.

Biology

BPI (2001) states that *D. neobrevipes* is a common pest of bananas. In the Philippines, high *D. neobrevipes* populations are found on the fruit and pseudostem of banana plants in the summer

(PCARRD 1988). Most work concerning the biology of *D. neobrevipes* has been done on pineapple (Kessing and Mau 1992). There appear to be no studies of its biology on bananas.

Dysmicoccus neobrevipes is a long-tailed mealybug. Long-tailed mealybugs reproduce sexually and produce live young or nymphs called ‘crawlers’ after mating (Kessing and Mau 1992). Adult females appear predominantly grey in colour. Their bodies are brown to greyish orange, but take on a greyish appearance in combination with the waxy exudation that covers them (Kessing and Mau 1992). The body is broadly oval and measures 1.5 mm long by 1.0 mm wide. The back is heavily coated with tiny tufts of white mealy wax. Short filaments of wax extend from around the margin of the entire body. Lateral or side wax filaments are usually less than one quarter as long as the breadth of the body and the tail filaments are half the body length.

Dysmicoccus neobrevipes only reproduces sexually (Ito 1938; Beardsley 1965; Rohrbach et al 1988). No eggs are laid as the eggs hatch inside the mother. The young emerge from the female mealybug as fully-developed first instar crawlers or nymphs. The crawler stage is the primary self-dispersal stage (Rohrbach et al 1988). Crawlers move about actively for a short period of time, no more than a day, and may be dispersed on to other plants up to several hundred yards by wind blowing infested leaves (Rohrbach et al 1988). In addition, ants may transport them short distances. Williams (2004) states that due to the small size and ‘cryptic’ nature of mealybugs, long-range dispersal often occurs through human transport of infested plant material from one area to another. Crawlers cannot survive longer than 24 hours without feeding (North Carolina State University 2005).

Females undergo three nymphal instar stages (moults) before reaching maturity; the nymphal instar stages last for 11–23 days, 6–20 days and 7–28 days, respectively (Kessing and Mau 1992), or an average of 8–14 days (Ito 1938). The total nymphal instar period varies from 26–52 days, averaging about 35 days (Kessing and Mau 1992). When the adult female emerges, there is a period of about 25 days before it produces its first larvae or crawlers (Kessing and Mau 1992). During this period the female is mated by males. Further mating can take place at any time after the maturation of the female. The female then produces nymphs for a period of about 30 days (Kessing and Mau 1992). Females die about four days after they cease producing young (Ito 1938; Kessing and Mau 1992). Each female can produce up to 350 crawlers (Ito 1938), but some produce up to 1000 young (Kessing and Mau 1992). Unmated females live for an average length of 148 days, while mated females live for an average of 95 days (Ito 1938). Duration of female adult life varies from 48–72 days, averaging about 61 days (Kessing and Mau 1992). The lifespan from first instar to adult death varies from 59–117 days, averaging 90 days (Kessing and Mau 1992).

Males moult four times before reaching the winged adult stage: the first nymphal instar stage lasts for 11–19 days, the second for 7–19 days, the third for 2–7 days and the fourth for 2–8 days (Kessing and Mau 1992), or an average of 3–13 days (Ito 1938). The total nymphal instar period varies from 22–53 days (Kessing and Mau 1992). Feeding is limited to the first and second instars, which together last for about 20 days. The second, third and fourth moults of the male take place inside a waxy cocoon during a period of about 12 days. When the adult male emerges from this cocoon, it is a fragile insect about 1 mm long with a pair of membranous wings. It has no mouthparts, and lives for only 2–7 days in order to mate (Ito 1938; Kessing and Mau 1992).

Dysmicoccus neobrevipes is tended by *Pheidole megacephala* (Big-headed ant) in pineapple fields in Hawaii. This ant greatly encourages the mealybug by interfering with its natural enemies, and maintains the health of the mealybug colony by removing excess honeydew (Beardsley et al 1982). Ants move mealybugs from one plant to another, and control of mealybugs depends on control of the ants (Carter 1962; McEwen et al 1979; Beardsley et al 1982). *Pheidole megacephala* is common in eastern and northern Australia (Shattuck 1998) and has been reported in all other states and territories of Australia except South Australia (CSIRO–AFFA 2004e).

Dysmicoccus neobrevipes is the principal vector of pineapple wilt disease (Beardsley 1965; McEwen et al 1979; Rohrbach et al 1988), which appears to be caused by a virus (Carter 1963). This is the most serious type of damage to pineapples and is the principal cause of crop failure in Hawaii (Kessing and Mau 1992). It can cause complete loss of pineapple crops if not controlled (Beardsley 1993). Fortunately, this disease has been controlled for the last three decades by routine ant control (Kessing

and Mau 1992). However, it may once again become prevalent if mealybugs are not continually suppressed by limiting ant populations (Kessing and Mau 1992).

Dysmicoccus neobrevipes is implicated as causing a physiological reaction known as ‘green spot’ on pineapples (Beardsley 1965).

There are no reports of *D. neobrevipes* acting as a banana disease vector.

12.2 *Nipaecoccus nipae*

Scientific name

Nipaecoccus nipae Maskell 1893 [Hemiptera: Pseudococcidae]

Synonyms

Dactylopius nipae Maskell 1893; *Dactylopius pseudonipae* Cockerell 1897; *Ripersia serrata* Tinsley 1900; *Pseudococcus nipae* Cockerell 1902; *Pseudococcus pseudonipae* Fernald 1903; *Ceroputo nipae* Lindinger 1924; *Pseudococcus magnoliae* Hambleton 1935; *Ripersia nipae* Gómez-Menor Ortega 1937; *Nipaecoccus nipae* Šulc 1945; *Nipaecoccus pseudonipae* Beardsley 1960.

Common names

Spiked mealybug; buff coconut mealybug; nipa mealybug;, avocado mealybug; sugarapple mealybug

Hosts

Nipaecoccus nipae is polyphagous and has been recorded on 80 hosts from 43 plant families. These hosts include commercial fruit, non-commercial plants, ornamental plants including palms and orchids.

The following host list was extracted from CAB International (2006):

Aglaonema sp; *Alchornea* sp; *Aloe ciliaris; Alpinia nutans; Ananas sativus; Annona muricate; Annona reticulata; Annona squamosa; Anthurium* sp; *Anthurium acaule; Anthurium andreanum; Arecaceae* (plants of the palm family*); Areca* sp; *Areca bambu; Arecastrum romanzoffianum; Arenga sacharifera; Artocarpus altilis; Artocarpus communis; Asparagus sporangia; Barringtonia speciosa; Bombacopsis* sp; *Calophyllum calaba; Calyptrogyne; Canna* sp; *Carica papaya; Carludovica palmata; Castilloa* sp; *Chamaedorea* sp; *Chamaerops excelsa; Chrysobalanus icaca; Citrus* sp; *Coccoloba uvifera; Cocos nucifera; Cola acuminata; Costus; Curatella Americana; Curculigo recurvata; Dracaena* sp; *Dracaena draco; Elaeis guineensis; Ficus benghalensis; Ficus carica; Ficus elastica; Gossypium* sp; *Gronophyllum* sp; *Heliconia* sp; *Herrania nitida; Inga* sp; *Ipomea batatas; Kentia* sp; *Kentia elmoriana; Kentia forestriana; Kentiopsis* sp; *Latania bourbonica* sp; *Livingstonia inensis; Luehea seemannii; Machaerium; Mangifera indica; Manihot esculenta; Monstera* sp; *Morus* sp; *Musa* sp; *Nypa fruticans; Olea* sp; *Pandanus* sp; *Persea Americana; Petrea erecta; Philodendron pinnatifidum; Pimenta dioica; Pithecellobium lomeratum; Pleurothallis* sp; *Pritchardia* sp; *Psidium guajava; Psidium* sp; *Ptychosperma* sp; *Rhapis humilis; Rhus* sp; *Roupala montana; Sabal* sp; *Solanum tuberosum; Sterculia urens; Straussia* sp; *Strelitzia* sp; *Strelitzia augusta; Styrax ferrugineus; Sweetia dasycarpus; Tetracera portobellensis; Theobroma bicolour; Theobroma grandiflora; Theobroma ubincatum; Tillandsia* sp; *Urera* sp; *Vismia latifolia; Vitis* sp; *Yucca elephantipes; Zingiber* sp.

Distribution

Table 12.2 lists the countries where *N. nipae* has been recoded.

Table 12.2 Distribution list for *Nipaecoccus nipae*
Source: Ben-Dov et al (2006d); and, for the Philippine, Lit et al (2006)

Algeria	England	Poland
Antigua	Georgia	Philippines
Argentina	Grenada	Portugal
Bahamas	Guadeloupe	Puerto Rico
Barbuda	Guatemala	Russia
Belize	Guyana	Saint Lucia
Bermuda	Hawaii	San Marino
Brazil	Hungary	Scotland
Canary Islands	India	South Korea
China	Indonesia	Spain
Colombia	Italy	Surinam
Costa Rica	Jamaica	Tobago
Cuba	Madeira Islands	Trinidad
Czech Republic	Mexico	Turkey
Dominica	Nicaragua	US Virgin Islands
Dominican Republic	Pakistan	USA
Ecuador	Panama	Venezuela
El Salvador	Peru	Vietnam

Plant parts affected

Fruits/pods, growing points, leaves and stems (CAB International 2006).

Biology

Adult female *N. nipae* are 3.5 mm long, flattish and oval. They are salmon-pink to dark-red in colour, with distinctive dorsal and marginal white or yellow wax cones, which create a satellite appearance. Male *N. nipae* produce small (about 2 mm long) waxy filamentous cocoons on the foliage, which are often present in large numbers and more common than females. *N. nipae* reproduces sexually, but its biology and ecology are poorly known.

Immature and adult females are readily carried on plants and plant produce and may be injurious when introduced to new geographical areas where they have no natural enemies.

Nipaecoccus nipae has become a pest of avocados and guavas in Hawaii, Bermuda and Puerto Rico. Ant-attended infestations of *N. nipae* have been recorded causing damage to coconut plantations in Guyana, together with the coconut scale *Aspidiotus destructor*. *N. nipae* is also a pest of bananas, ornamental palms and orchids.

Damage caused by *N. nipae* on ornamental plants, fruit or cut flowers may result in yellowing of the foliage and reduced vigour of the host due to the depletion of plant sap.

Heavy infestations of *N. nipae* are conspicuous because of the presence of distinct waxy adult females, numerous waxy male tests and sooty mould growing on the honeydew. They generally occur on the undersides of the foliage but have also been observed to affect the fruiting stage of their hosts (CAB International 2006).

12.3 *Pseudococcus jackbeardsleyi*

Scientific name

Pseudococcus jackbeardsleyi Gimpel and Miller 1996 [Hemiptera: Pseudococcidae]

Synonyms

Pseudococcus jackbeardsleyi was previously known in the Philippines and Hawaii as *P. elisae,* the banana mealybug (Beardsley 1986; Lit and Calilung 1994; Mau and Kessing 1993b). However, Gimpel and Miller (1996) discovered that the species previously identified as *P. elisae* actually represented two cryptic species, and changed this description to *P. jackbeardsleyi* and *P. elisae*. As a result, the literature before this date is full of misidentification and inaccurate information regarding these two species.

Ben-Dov et al (2005c) provides a complete list of synonyms.

Common name

Jack Beardsley mealybug.

Hosts

Pseudococcus jackbeardsleyi is polyphagous and has been recorded on 153 hosts from 51 plant families. These hosts include commercial fruit, vegetable and tree crops, non-commercial plants, ornamental plants and a number of weeds.

The following host list was taken from Ben-Dov et al (2005c), Williams (2004) and Biosecurity New Zealand MAF, Wellington, New Zealand (2005b).

Acacia sp.; *Acalypha wilkesiana; Acanthocereus; Acrotrema costatum; Aeschynomene americana* (forage legume, American jointvetch); *Agave sisalana* (sisal); *Aglaonema commutatum; Aglaonema simplex; Aglaonema* sp.; *Alpinia purpurata* (red ginger, gingerlily); *Alpinia* sp.*; Ananas comosus* (pineapple); *Annona cherimola* (custard apple, cherimola); *Annona muricata* (sour sop); *Annona squamosa* (sweet sop, sugar apple); *Annona* sp.; *Anthurium* sp. (tropical flower); *Apium graveolens* (celery); *Aporusa aurea; Aralia* sp.*; Begonia* sp.*; Bidens bipinnatea; Blighia sapida* (akee apple); *Bougainvillea* sp.*; Cajanus cajan* (pigeon pea); *Cajanus indicus* (pigeon pea); *Capsicum annuum* (sweet pepper); *Capsicum fructescens* (sweet pepper); *Capsicum* sp.*; Carica papaya* (papaya); *Cattleya* (orchid); *Cereus peruvianus* (cactus); *Cereus* sp.; *Chamaesyce; Chrysophyllum cainito* (caimito); *Citrus aurantiifolia* (Mexican lime); *Citrus paradisi* (grapefruit); *Citrus* sp.; *Coccinia grandis* (scarlet gourd); *Cocos* sp*.; Codiaeum* sp.*; Codiaeum variegatum* (croton); *Coffea arabica* (coffee); *Coleus* sp.*; Conocarpus erectus* (buttonwood); *Cordia curassavica; Coryphanta cubensis; Cosmosroton bipinnatus* (garden cosmos); *Cucumis melon* (oriental melon); *Cucurbita pepo* (zucchini); *Cucurbita* sp. (ornamental gourd); *Cycnoches* sp.*; Cymbopogon citratus* (lemon grass); *Dendrobium tortile* (orchid); *Dendrobium* sp. (orchid); *Dieffenbachia* sp.; *Dracaena* sp. (Caiman lizard); *Eucalyptus* sp.; *Eugenia* sp.*; Eupatorium odoratum* (Siam weed); *Euphorbia* sp. (spurges); *Fernaldia* sp.*; Ficus decora* (rubber plant); *Ficus tricolor; Ficus* sp.*; Gardenia jasminoides* (cape jasmine); *Gossypium barbadense* (bourbon cotton); *Gossypium* sp. (cotton); *Haematoxylum campechianum* (campeachy wood); *Heliconia* sp. (cut flower); *Hibiscus cannabinus* (kenaf); *Hibiscus esculentus* (okra); *Hibiscus* sp.*; Hoya carnosa* (ornamental flower plant, wax plant); *Hura crepitans* (sandbox tree); *Ipomoea batatas* (sweet potato); *Ipomoea* sp.*; Iris* sp.*; Jatropha curcas* (Barbados nut); *Jatropha* sp.*; Lantana camara* (lantana); *Litchi chinensis* (lychee); *Lycopersicon esculentum* (tomato); *Macadamia* sp.*; Mangifera indica* (mango); *Manihot esculenta* (manioc, cassava); *Melocactus* sp. (cactus); *Melochia tomentose; Mentha* sp. (mint); *Moringa oleifera* (drumstick, *ben-oil* tree); *Mormolyca balsamina; Morus* sp. (mulberry tree); *Mucuna* sp. (velvet beans); *Musa x paradasiaca* (banana); *Musa sapientum* (banana); *Musa* sp.*; Nephelium lappaceum* (rambutan); *Nephelium* sp.*; Nephrolepis* (sword fern); *Nerium oleander* (Mediterranean shrub, oleander); *Ocimum*

sp.; *Salvia* sp.; *Paphiopedilum* (lady's slipper orchid); *Pelargonium* sp.; *Persea* sp.; *Phaeomeria* sp.; *Phaseolus limensis* (lima bean); *Physalis peruviana* (cape gooseberry); *Physalis pubescens* (ground cherry); *Piper nigrum* (black pepper); *Plumeria* sp.; *Psidium guava* (guava); *Psidium* sp.; *Pueraria javanica; Punica granatum* (pomegranate); *Rhipsalis mesembrianthemoides; Rumex* sp.; *Sechium edule* (chayote); *Solanum melongena* (eggplant); *Solanum tuberosum* (potato); *Solanum* sp.; *Spondias* (purple mombin); *Tamarindus indica* (Indian tamarind); *Tamarindus* sp.; *Theobroma cacao* (cocoa); *Trichosanthes cumumesina* (snake gourd); *Vitis* sp. (grapes); *Yucca* sp.; *Zea mays* (maize); *Zingiber officinale* (ginger).

Plant parts affected

Leaves and fruits (Gimpel and Miller 1996).

Distribution

Pseudococcus jackbeardsleyi is found throughout Central and South America, the Hawaiian Islands, a few countries in southern Asia, including the Philippines (Williams and Watson 1988), and some Pacific island countries. Records referring to *P. elisae* in the Hawaiian Islands and southern Asia are usually misidentifications of *P. jackbeardsleyi* (Gimpel and Miller 1996). Table 12.3 lists the countries where *P. jackbeardsleyi* has been recorded.

Williams (2004) states that, in southern Asia, *P. jackbeardsleyi* is easy to identify because it is the only *Pseudococcus* species in this area with distinct markings on its body. Work by Gimpel and Miller (1996) enabled them to determine that many specimens previously identified as *P. elisae* in Asia were in fact *P. jackbeardsleyi*. *P. elisae*, the Banana mealybug, has a localised distribution and occurs exclusively in Central America, northern South America and Florida (Mau and Kessing 1993b; Williams 2004, Biosecurity New Zealand MAF, Wellington, New Zealand 2005b).

Table 12.3 Distribution list for Pseudococcus jackbeardsleyi

Source: Ben-Dov et al (2002, 2005b) and Biosecurity New Zealand MAF, Wellington, New Zealand (2005b)

Aruba	Grenada	Papua New Guinea
Bahamas	Guatemala	Peru
Barbados	Guyana	Philippines
Belize	Haiti	Puerto Rico and Vieques Island (Puerto Rico)
Bolivia	Hawaiian Islands (Hawaii)	Singapore
Brazil	Honduras	St. Martin
Brunei	Indonesia	Taiwan
Canada	Jamaica	Thailand
China	Kiribati	Trinidad and Tobago
Colombia	Malaysia	Turks and Caicos Islands
Costa Rica	Maldives	Tuvalu
Cuba	Martinique	U.S. Virgin Islands
Dominican Republic	Mexico	United States of America (Florida, Texas)
El Salvador	Montserrat	Venezuela
Federated States of Micronesia (Caroline Islands)	Nicaragua	Vietnam
Galapagos Islands	Panama	
	Panama Canal Zone	

Biology

No information about the biology of *P. jackbeardsleyi* on bananas could be found. However, Mau and Kessing (1993) note that the life histories of all mealybugs are quite similar. The following information is from Mau and Kessing (1993b).

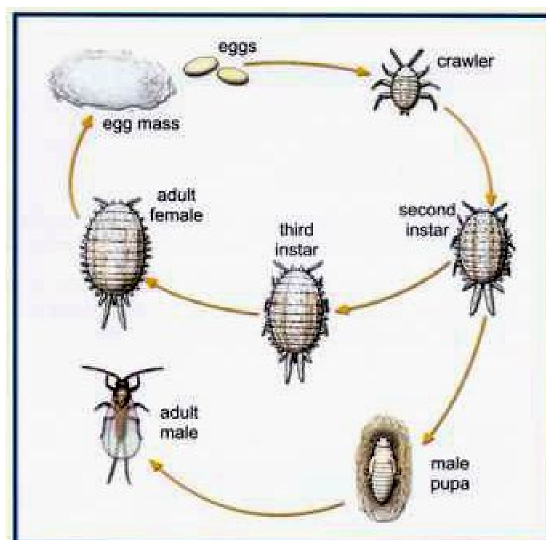


Figure 12.1 Short-tailed mealybug lifecycle
Source: South Australian Research and Development Institute[3]

The crawlers are pale yellow and oval-shaped with six legs. Once they find a suitable feeding site, they inject their stylets or feeding tubes into the plant and usually do not move. They can survive only about a day without food (North Carolina State University 2005). They produce a white waxy material which creates small filaments around the edge of their bodies. This gives them their 'mealy' appearance.

First instar males are similar to first instar females, but second instar males form a waxy sac and pass through two more non-feeding instars (the pre-pupa and pupa) before becoming winged adults (see Figure 12.1). Adult females remain the same in appearance and just grow larger. After mating, females lay eggs for 1–2 weeks and die once egg production has stopped.

Short-tailed mealybugs in general have four female and five male instars before becoming adults. Adult female mealybugs are slow moving. Adult males resemble small flies and have wings for dispersal, but their mouthparts are not functional and they cannot feed (North Carolina State University 2005). On emergence, the adult males disperse and it is assumed that most mealybug males locate females using a pheromone. Males can often be seen in flight early in the morning or late in the day when winds are generally calm. After mating, the male adults usually survive for no more than a day. Mealybugs have from one to nine generations a year, depending on the weather conditions and individual species.

The relationship between *P. jackbeardsleyi* and ants is unknown at present. However, many mealybug species are attended by ants that feed on the honeydew they produce and help to protect them from natural enemies.

There are no reports of *P. jackbeardsleyi* acting as disease vectors.

[3]http://www.sardi.sa.gov.au/pages/entomolo/pdf/mbug_id.pdf

12.4 *Planococcus minor*

Scientific name

Planococcus minor (Maskell 1897) [Hemiptera: Pseudococcidae]

Synonyms

Dactylopius calceolariae minor Maskell 1897
Pseudococcus calceolariae minor Maskell 1897
Planococcus pacificus Cox 1981

Common names

Pacific mealybug; passionvine mealybug.

Hosts

Planococcus minor is highly polyphagous and feeds on more than 250 reported wild and commercial host plants in nearly 80 families. Banana, citrus, cocoa, coffee, corn, grape, mango, potato and soybean are among the more notable agricultural crops that may be affected by this pest (Ben-Dov et al 2006e; Venette and Davis 2004). Venette and Davis (2004) provide a comprehensive list of all reported host plants of this mealybug.

Plant parts affected

For the listed mealybug species, the plant parts affected include leaves and fruit (Bentley et al 2003; CAB International 2006; Peña and Mohyuddin 1997; Srivastava 1997; IES (2006).

Distribution

Table 12.4 lists the countries where *Planococcus minor* has been recorded.

Table 12.4 Distribution list for *Planococcus minor*

Source: Ben-Dov et al (2006e) for all listed countries

American Samoa	French Polynesia (Austral Islands, Society Islands)	Mexico
Antigua and Barbuda	Grenada	Myanmar
Argentina	Guadeloupe	New Caledonia Niue
Australia (New South Wales, Queensland, South Australia, Northern Territory)	Guatemala	Papua New Guinea
Bangladesh	Guyana	Philippines
Bermuda	Haiti	Saint Lucia
Brazil	Honduras	Seychelles
British Indian Ocean Territory	India (Karnataka)	Singapore
China (Taiwan)	Indonesia (Irian Jaya, Kalimantan, Sumatra)	Solomon Islands
Colombia	Jamaica	Surinam
Cook Islands	Kiribati	Thailand
Costa Rica	Madagascar	Tonga
Cuba	Malaysia	Trinidad & Tobago
Dominica	Mauritius (Rodriques Island)	US Virgin Islands
Ecuador (Galapagos Islands)		Uruguay
Fiji		Vanuatu
		Western Samoa

Biology

Venette and Davis (2004) provide a comprehensive biology of *P. minor* and the following information has been summarised from their document.

There has been no detailed biological study of this species on bananas in the Philippines. With the exception of a few species such as *Planococcus citri*, details about life stages of many mealybugs, particularly *P. minor*, are not well known. However, *P minor* is a short-tailed mealybug like *P. jackbeardsleyi* above and therefore, reproduces sexually, laying eggs after mating.

Planococcus citri has 4–8 generations annually, and within *Planococcus* species, there are typically four instars for females and five for males (Williams 1985). Development rate and the number of generations is highly variable and determined by several factors, including plant host selection and feeding site (as these relate to nutrition), temperature, population density, and the presence of predators (McKenzie 1967; Williams 1985).

Population density may also vary depending on the presence of ants (several genera) that are known to have an association with *Planococcus* species. Ants have been observed feeding on the honeydew excretions of mealybugs and protecting this important food source from predators. Ants may also play a role in mealybug dispersal. Other ants may be predators. Ants may also be selective of the mealybug species they tend, when multiple species are present. Mealybug populations closely associated with ants tend to be larger than non-tended populations of the same species (McKenzie 1967).

Planococcus minor females produce between 65–425 eggs on varying hosts in the lab. The pre-oviposition period ranged from 8–12 days, and the incubation period lasted approximately three days. The time to complete one generation ranged from 31–50 days. The median development time for males was slightly longer than for females (Table 12.5) (Venette and Davis 2004). *Planococcus minor* is a phloem feeder which may allow it to act as a vector of plant diseases (Williams 1985).

Table 12.5 Median development time (days) for instars on various hosts in the laboratory

Source: Venette and Davis (2004)

Larval instar	Male	Female
First	8.22	8.01
Second	15.18	13.29
Third	17.67	17.49
Fourth	21.36	NA

Appendix 13. Spider mites

Introduction

A general introduction on tetranychid biology is included because it provides much more detailed information on the life history, behaviour and natural history of this group than is available for the five species being assessed in this IRA. While there are some differences in the biology of each of these five pests, there are numerous generalisations that can be made about the biology of the family. The following general description of tetranychid biology is derived from Macgregor (1950), Helle and Sabelis (1985), Lui and Lui (1986), Cayme and Gapasin (1987) and Bell et al (2005).

Tetranychid mites are small mites with adults under half a millimetre in length. Despite their common name of red spider mite, the body colour, even within species, can vary from green or white through to yellow, orange and red. For instance, diapausing individuals are white or yellow/orange and large males may be white (Helle and Sabelis 1985). Many species of spider mites (alternative names: red spiders, spinning spiders) are important pests and cause damage or loss to agricultural crops, ornamentals and forest plants (McGregor 1950; Helle and Sabelis 1985). The biology and lifecycle of some pest tetranychids have been studied in detail and much of the information available refers to these pest species.

Mating behaviour is described in detail by Helle and Overmeer (1973). Males compete for females and begin their mating behaviour by guarding female deutonymphs. Fertilisation may take place after eclosion of the female or later. All tetranychids undergo incomplete metamorphosis and develop through eggs, larvae, nymphs and adults, but a change from one stage to the next generally involves an increase in size. There is no pupal stage and the main morphological change is from the six-legged larvae to the eight-legged nymph. Eggs from 110–150 µm in length are laid singly in rows or small clusters on the surface of the food plant, or inside web nests (Rudd 1997). These eggs can be white, green, orange, red or brown in colour and hatch in a few days, giving rise to larvae (Helle and Sabelis 1985). Females each produce around 155 eggs throughout their lifetime.

New colonies are established by dispersing mated or unmated females. Although unmated females are only able to lay eggs that give rise to male offspring, such founder females, if they live long enough, can mate with their sons and produce female offspring.

Both male and female spider mites pass through one larval and two nymphal stages before becoming an adult. Temperature is an important influence on the spider mite lifecycle and it also affects the temporal and spatial distribution of mites (Bounfour and Tanigoshi 2001). While the lifecycle from egg to adult varies between genera and species, it generally ranges from 7–14 days depending on temperature, being shorter at high temperatures. The longevity of adult tetranychid mites can also vary with host plant. The survival time of adult females of two tetranychid species, including *Tetranychus urticae* (Zhang et al 2001) varied from 4–30 days, depending on host plant, host quality and temperature (Bounfour and Tanigoshi 2001). For example, at 15 °C, *T. urticae* survived for 22 days (Bounfour and Tanigoshi 2001).

Tetranychid mites can be found damaging bananas in plantations and also in greenhouses holding seedling plants (Ahn et al 1990). They are abundant in banana plantations in the Philippines (H Krishna, Senior Scientist, Fresh Product Protection Crop and Food Research, Palmerston, New Zealand, pers comm 8 June 2006). Populations reach their peak in temperate climates during summer, but where winters are mild, as in the tropics, spider mites remain active throughout the year and there is no pause in development.

Outbreaks can be affected by other factors apart from climate. For instance, fungal diseases can inhibit spider mite outbreaks, while dust on leaves can often facilitate population growth (Kennedy and Smitley 1985). In warm climates there can be as many as 26 generations produced each year. In colder temperate conditions, spider mites survive the low temperatures by going into diapause (overwintering) as either eggs or adult females. Diapause is initiated by decreasing day length, lower

temperatures and an unfavourable food supply. During diapause individuals may change colour, often becoming darker and they may move to cracks and crevices or other protected spaces. Eggs remain on the host plant (Jeppson et al 1975), but may also be laid in large numbers in protected areas, such as cracks on the trunk of the host plant. Diapause is broken by increasing day length and temperatures.

All active stages of tetranychid mites are phytophagous, feeding on plant tissue or fluids. They feed by puncturing plant tissues with their stylet-shaped mandibles, which form a straw-like tube through which they remove the cell contents by suction. Young leaves are preferred to old leaves and mites show higher fecundity on young leaves (Zhang et al 2001). Some species are polyphagous, including those that are the subject of this PRA, while others show preferences for particular hosts. An infestation usually begins on the outer parts of the plant and then spreads to include leaves, stems and fruit. Feeding activities remove chlorophyll and desiccate the plant, resulting partly in lower humidities that are detected by the mites and induce them to move upwards. Mites feeding on fruit tend to seek out protected spaces, such as the cavities between the fingers of banana fruit within a bunch. Damage caused by mites is evident by bleaching of the leaves in a characteristic blotchy or stippled pattern. Discolouration and stunting of fruit can also occur, and severely attacked plants can be completely defoliated or killed.

Nymphs and adults of many spider mites, especially species of *Tetranychus*, spin webbing that resembles a spider's web on or between leaves. Web production seems to be enhanced under intense or continuous light (Gerson 1985). With heavy infestations, the whole plant may become shrouded with dense webbing visible to the naked eye. Webbing is believed to have several functions. For many species, webbing is used to protect their eggs by:

- securing them to the substrate
- providing some protection from predators and
- maintaining a uniform level of humidity for the eggs.

Some species also use silk during mating and some spin silk threads as they walk. Such threads may have a direction-finding role and act as a 'lifeline' if mites are dislodged from the host. Finally, webbing is used by many species to produce a web nest in which the spider mites secrete themselves. These nests are thought to provide protection from certain predators and from adverse environmental conditions such as strong winds and rain. The web nests also provide protection against acaricide sprays and dusts (Gerson 1985).

Application of acaricides appears to encourage migration by inducing 'spin down' by mites. Mites coming in contact with acaricides drop from the host plant and hang by a silk thread (Walter and Proctor 1999). Eggs are sometimes laid inside the webbing, where they are protected from chemicals (Rudd 1997). There is some evidence that strongly-webbing species, such as species of *Tetranychus,* can displace other plant-feeding mites.

Although these mites are small, around half a millimetre in length with a walking speed estimated at between 5 cm – 6 m per hour, they have dispersal mechanisms which allow them to travel long distances. They are readily blown by air currents, becoming part of the aerial plankton.

Under conditions of high density and plant damage, the mites undergo a change in behaviour, transforming from a feeding or reproductive phase to a dispersal phase. A proportion of the newly fertilised adult females will disperse regardless of the population density on the natal leaf (Walter and Proctor 1999), but the majority of ballooning individuals are recently emerged females, and can be either mated or unmated. Females and sometimes nymphs move to the periphery of the plant where they either drop (spin down) off the host on a thread of silk when the air is still or, if there is a wind, raise the tip of their bodies and release a thread which enables them to be caught by wind currents (ballooning) in the same manner as juvenile spiders. They respond to wind by facing away from the source of light, raising their first pair of legs and body surfing on the wind current (Kennedy and Smitley 1985).

Other factors can induce a change to a dispersal phase in the mites, such as the application of an acaricide, which may have an anti-feeding effect. New colonies of tetranychid mites are known to start primarily with the arrival of dispersing pre-reproductive females onto new substrates (Kennedy and

Smitley 1985). Norambuena et al (2000) document ballooning *Tetranychus lintearius* Dufour, 1832 travelling long distances of from 6–1000 m (mean = 157 m) from gorse in Hawaii and Chile.

Establishment commonly first takes place along the direction of prevailing wind. Wind is frequent in plantations on Mindanao as the island is in an area subject to typhoons, and severe weather of this type seems to occur every five years (Alojado and Padua 2000). If landscape features interrupt the flow of air, spider mite populations can establish more easily because dispersal is interrupted. Rapid spread can follow establishment because of the high mobility of mites.

Tetranychid mites can be controlled on crops by the application of pesticides as dusts or sprays. Two applications a fortnight apart is normal, but this varies with crop and season. However, spider mites are known to readily develop resistance to acaricides, possibly because favourable mutations become fixed rapidly in haploid males (Crozier 1985; Walter and Proctor 1999). Resistance contributes to outbreaks, especially if natural predators are adversely affected (Chapman and Martin 2003).

Methyl bromide fumigation, applied at the standard rates used for insects, is not a fully effective quarantine control method for mites as the eggs are particularly resistant at low temperatures (Mizobuchi et al 1997; Bell et al 2003). Because of the tolerance displayed by mite eggs towards fumigation, rates will need to be 50% higher than those recommended for insect control (Bennett 2003). An alternative method and one that achieves total mortality is the application of high concentrations of CO_2 (65%, 80% or 95%) for one to three days at 0 °C, followed by 18 days at 0 °C in air, 8% CO_2 or 20% CO_2 and 5% O_2 (Zhou and Mitcham 1998). Hot water treatment at temperatures between 44 °C and 54 °C for 211 minutes achieved 99% mortality of non-diapausing *T. urticae* on persimmons (Lester et al 1997). Diapausing individuals are less tolerant of high temperatures and mites can survive cool storage at 20 °C or less for several months (Lester et al 1997).

Comments on *Oligonychus, Raoiella* and *Tetranychus* species

The following four species of spider mites are of quarantine significance to the whole of Australia and occur on bananas in the Philippines:

13.1 *Oligonychus orthius* Rimando 1962
13.2 *Oligonychus velascoi* Rimando 1962
13.3 *Raoiella indica* Hirst 1924
13.4 *Tetranychus piercei* McGregor 1950.

The following species is of quarantine significance to Western Australia:

13.5 *Tetranychus marianae* McGregor 1950

All five spider mite species included in this assessment are polyphagous, but some *Oligonychus* species are predominately grass-feeding, although they have been associated with a range of plant hosts, including bananas and corn (Corpuz-Raros 1989; Bolland et al 1998). Other *Oligonychus* species feed on dicotyledons. *O. orthius* has also been associated with sugarcane (Corpuz-Raros 1989; Bolland et al 1998), while *O. velascoi* has been found on coconut (Cayme and Gapasin 1987; Corpuz-Raros 1989; Bolland et al 1998). The known host range of *R.indica* comprises more than 25 plant species, including commercial palms and plants within the Musaceae and Zingiberaceae families (Pena et al 2006). Both *T. marianae* and *T. piercei* have a known host range comprising more than 30 plant species and includes bananas, papayas and sweet potatoes (Corpuz-Raros 1989; Bolland et al 1998). Although there are differences in biology between the genera *Oligonychus, Raoiella* and *Tetranychus*, all five species are assessed together except where otherwise indicated, with assessments made for *T. piercei* as the most damaging species.

Other information relevant to the biology of the five species of tetranychid mites on the pathway is available in the following individual datasheets.

13.1 *Oligonychus orthius*

Scientific name

Oligonychus orthius Rimando 1962 [Acari: Tetranychidae]

Synonyms

None

Common name

Spider mite

Hosts

The following host list was taken from Ehara (1969), Lee et al (1987), Corpuz-Raros (1989) and Bolland et al (1998).

Brachuaria mutica (para grass); *Imperata cylindrica* (alang-alang); *Miscanthus sinensis* (Japanese pampas grass); *Musa sapientum* (dwarf Cavendish); *Musa* sp.; *Pandanus odoratissimus*; *Saccharum officinarum* (sugarcane); *Saccharum spontaneum* (tigergrass); *Sorghum bicolour* (sorghum); *Sorghum* sp.; *Wedelia biflora*; *Zea mays* (maize).

Distribution

Table 13.1 lists the countries where *O. orthius* has been recorded.

Table 13.1 Distribution list for *Oligonychus orthius*

References: Bolland et al (1998), Ehara (1969), Copruz-Raros (1989) and Wang (1981)

China	Korea	Taiwan
Japan (Okinawa Is.)	Philippines	Thailand

Biology

There is very little published information on the biology of *O. orthius.*

The male is distinct in that the distal end of the aedeagus is obtusely, rather than sharply, curved. The species is close to *O. zeae* (McGregor 1950) and some other species, except that the terminal shaft of the aedeagus is shorter (Jeppson et al 1975).

Many species of *Oligonychus* are grass-feeding, but they can be fairly polyphagous. Beard et al (2003) note that *'Australia is fortunate because most of these grass-feeding specialists do not occur here'*, as they have the potential to become significant pests on sugarcane and other grass crops such as rice, sorghum and exotic pasture and native grasses.

13.2 *Oligonychus velascoi*

Scientific name

Oligonychus velascoi Rimando 1962 [Acari: Tetranychidae]

Synonym

None

Common name

Coconut spider mite

Hosts

The following host list was taken from Cayme and Gapasin (1987), Ehara and Wongsiria (1975), Corpuz-Raros (1989), Charernsom (2003) and Bolland et al (1998).

Adonidia merrilli; Caryota cumingii; Cocos nucifera (coconut); *Corchorus* sp. (jute); *Corypha elata* (buri palm); *Digitaria* sp. (couch grass); *Mangifera indica* (mango); *Musa* sp.; *Pennisetum purpureum* (napier grass); *Ptychosperma macarthurii* (ornamental palm); *Zea mays* (maize).

Distribution

Table 13.2 lists the countries where *O. velascoi* has been recorded.

Table 13.2 Distribution list for *Oligonychus velascoi*

Country	Reference
Philippines	Bolland et al (1998)
Thailand	Bolland et al (1998)

Biology

No published information is available concerning the biology of this mite on bananas in the Philippines, but Cayme and Gapasin (1987) studied the biology, host range and natural enemies of *O. velascoi* on coconuts in the Philippines.

Cultures were established on both attached and detached coconut leaflets in order to collect lifecycle data. The lifecycle of the mite from egg to adult was an average of 6.3 days on detached coconut leaflets and 6.9 days on attached leaflets, with a maximum of 23 days. Females laid an average of 26 eggs on detached leaflets and an average of 18 on attached leaflets. The incubation period of eggs was 3.9 days on detached leaves and 4.3 days on attached leaves. Hatching rates were 92% on detached and 87% on attached leaflets, respectively. Longevity was lower on attached leaflets, although mortality was also lower. The pre-oviposition period lasted 1–2 days and females generally continued to lay eggs for 20 days. Eggs were laid singly on the underside of leaves and the female produced a sticky material to cement the eggs to the leaves. Eclosion was completed in 4–10 minutes. Larvae feed immediately after hatching, but one to two days later they become quiescent as they moult into the next stage, the protonymph. This instar also feeds for 1–2 days before becoming quiescent for 1–2 days, followed by the second moult into the deutonymph instar. This instar is larger than the protonymph, and feeds frequently before undergoing another quiescent period, after which active adults emerge (Cayme and Gapasin 1987).

Although males are smaller, they are more active than females and immediately begin searching for a mate. Copulation can occur at any time, with the male crawling under the female and clasping her body with his first and second pair of legs. Mating lasts from several seconds to ten minutes. Although females normally mate only once, repeated matings can occur (Cayme and Gapasin 1987).

Oligonychus velascoi attacks coconut seedlings as well as mature palms and is fairly polyphagous. Some coconut palm varieties are more susceptible than others (Capuno and Pedro 1982). Feeding by spider mites results in discolouration of the leaves and dieback of leaf tissues, causing abnormal nut development and yield reductions of up to 25% (Cayme and Gapasin 1987).

Although these mites are usually found on leaves, they are able to move to other parts of the plants, including fruit, especially when populations are high (Pinese and Piper 1994; PHA 2004a).

The natural enemies of this mite in the Philippines include coccinellid beetles and phytoseid mites (Cayme and Gapasin 1987).

13.3 *Raoiella indica*

Scientific name

Raoiella indica Hirst 1924 [Acari: Tenuipalpidae]

Synonym

None

Common name

Red palm mite

Hosts

The following host list was taken from APHIS (2008), Welbourn (2007) and Pena et al (2006).

Acoelorraphe wrightii (everglades palm); *Adonidia merrilli* (christmas palm); *Aiphanes spp.* (ruffle palm); *Alpinia purpurata* (red ginger); *Areca catechu* (betel nut palm); *Areca* spp.; *Bactris plumeriana* (coco macaco); *Bismarckia nobilis* (bismarck palm); *Caryota mitis* (fishtail palm); *Chamaedorea* spp. (chamaedorea palm); *Cocos nucifera* (coconut palm); *Dictyosperma album* (princess palm); *Dypsis decaryi* (triangle palm); *Dypsis lutescens* (golden cane palm); *Elaeis guineensis* (african oil palm); *Etlingera elatior* (red torch ginger); *Heliconia psittacorum* (parrot flower); *Heliconia caribaea* (wild plantain); *Heliconia rostrata* (lobster claw heliconia); *Heliconia bihai* (macaw flower); *Licuala grandis* (ruffled fan palm); *Livistona chinensis* (chinese fan palm); *Musa acuminata* (plantain); *Musa balbisiana* (wild banana); *Musa corniculata* (red banana); *Musa x paradisiaca* (plantain); *Musa sapientum* (plantain); *Musa uranoscopus* (red-flowering banana); *Musa* spp.; *Ocimum basilicum*; *Pandanus utilis* (screw pine); *Phoenix canariensis* (canary island date palm); *Phoenix dactylifera* (date palm); *Phoenix reclinata* (senegal date palm); *Phoenix roebelenii* (pygmy date palm); *Pritchardia pacifica* (fiji fan palm); *Pseudophoenix sargentii* (buccaneer palm); *Pseudophoenix vinifera* (cacheo); *Ptychosperma elegans* (alexander palm); *Ptychosperma macarthurii* (macarthur palm); *Ravenala madagascariensis* (traveler's tree); *Rhapis excelsa* (bamboo palm); *Roystonea borinquena* (royal palm); *Strelitzia reginae* (bird of paradise); *Syagrus romanzoffiana* (queen palm); *Syagrus schizophylla* (arikury palm); *Washingtonia filifera* (fan palm); *Washingtonia robusta* (mexican fan palm); *Wodyetis bifurcata* (foxtail palm).

Distribution

Table 13.3 lists the countries where *R. indica* has been recorded.

Table 13.3 Distribution list for *Raoiella indica*

Country	Reference
Dominica	APHIS (2008)
Dominican Republic	APHIS (2008)
Egypt	Welbourn (2007)
Grenada	APHIS (2008)
Guadelope	APHIS (2008)
Haiti	APHIS (2008)
India	Welbourn (2007)
Iran	Welbourn (2007)
Israel	Welbourn (2007)
Jamaica	APHIS (2008)
Puerto Rico	APHIS (2008)

Malaysia	APHIS (2008)
Mauritius	Welbourn (2007)
Oman	Welbourn (2007)
Pakistan	Welbourn (2007)
Philippines	Welbourn (2007)
Puerto Rico	APHIS (2008)
Reunion	Welbourn (2007)
Saint Lucia	APHIS (2008)
Saudi Arabia	Welbourn (2007)
Sri Lanka	Welbourn (2007)
Sudan	Welbourn (2007)
Thailand	Welbourn (2007)
Trinidad and Tobago	APHIS (2008)
Venezuela	APHIS (2008)
Virgin Islands	APHIS (2008)
United Arab Emerits	Welbourn (2007)

Biology

The biology of *R. indica* is described in the review by Pena et al (2006). Under laboratory conditions between 24-26°C and 60% RH, females completed their development in 24.5 days and males in 20.6 days; adult longevity was 50.9 days for females and 21.6 days for males. Fertilized females produced an average of 22 eggs and virgin females 18.4 eggs. In Mauritius, the preoviposition period is 3 days in summer and 7 days in winter. Females lay an average of 2 eggs per day over an average oviposition period of 27 days providing an approximate fecundity of 50 eggs per female. Development times are: 6.1-6.5 days for eggs; 5.7-9.5 days for larvae; 5.4-6.5 days for protonymphs; and 4.1-10.5 days for deutonymphs. The lifecycle is completed in 21-33 days dependent on temperature, RH and host plant.

The within-plant distribution of *R. indica* is only known for coconut palm on which it primarily lives in groups ranging from 20 to 300 individuals (eggs, larvae, protonymphs and deutonymphs) on the underside of leaflets, particularly on lower leaves (Pena et al 2006).

All life stages of *R. indica* are red and adult females often exhibit black patches across their backs. *R. indica* can be distinguished from most Tetranychid spider mites by their red colour (including legs), long spatulate setae, flattened bodies, liquid droplets on dorsal body setae and absence of the webbing associated with many other spider mites. *R. indica* courtship behavior is unique among the Tetranychoidea. Males use their first pair of legs to attach to the posterior of female deutonymphs and an active colony will usually have males and female deutonymphs in tandem (Welbourn 2007).

13.4 *Tetranychus piercei*

Scientific name

Tetranychus piercei McGregor 1950 [Acari: Tetranychidae]

Synonym

Tetranychus manihotis Flechtmann 1981

Common name

Banana leaf mite

Hosts

The following host list was taken from Wang (1981), Corpuz-Raros (1989), Lee et al (1999), Masaki (2001), Welbourn (2005) and Bolland et al (1998).

Ageratum conyzoides (billy goat weed)*; Ageratum esculenta; Ageratum* sp.; *Arachis hypogaea* (ground nut); *Areca catechu* (betel nut); *Asarum blumei; Canavalia maritima* (beach bean); *Carica papaya* (papaya); *Cassia obtusifolia* (sickle pod); *Cassia tora* (sickle senna); *Clitoria ternatea* (butterfly pea); *Codiaeum variegatum* (croton); *Colocasia esculenta* (taro); *Curculigo orchioides* (orchid palm grass); *Dolichos lablab* (kidney bean); *Dracaena* sp.; *Elaeis guineensis* (African oil palm); *Glycine maximus* (soya); *Houttuynia cordata* (chameleon plant); *Ipomoea batatas* (sweet potato); *Lablab purpureus* (field bean); *Manihot esculenta* (manioc/cassava); *Manihot* sp.; *Morus alba* (mulberry); *Musa* sp.; *Musa sapientum* (= x paradisiaca) (dwarf Cavendish); *Musa textilis* (abacá); *Palmae (*plants of the palm family); *Pandanus amaryllifolius; Passiflora foetida* (love-in-a-mist); *Phaseolus vulgaris* (kidney bean); *Polygala paniculata; Prunus persica* (peach); *Psophocarpus tetragonolobus* (winged bean); *Pueraria montana* (kudzu); *Rhamnus crenatus; Ricinus communis* (castor oil); *Solanum melongena* (eggplant).

Plant parts affected

Mainly found on leaves, but also likely to be found on fruit when mite populations are high (Pinese and Piper 1994; PHA 2004a).

Distribution

The distribution is summarised in Corpuz-Raros (1989) and comprises only tropical and warm subtropical regions. Table 13.4 lists the countries where *T. piercei* has been recorded.

Table 13.4 Distribution list for *Tetranychus piercei*

Country	References
Cambodia	Waterhouse (1993)
China (Guangdong)	Liu and Liu (1986)
China (Jiangxi)	Wang (1981)
China (Zhejiang)	Wang (1981)
China (Hong Kong)	Ehara and Lee (1971)
China (Taiwan)	Ehara (1969); Ho et al (1997)
French Guiana	Flechtman and Etienne (2001); Bolland et al (1998); Flechtman and Knihinicki (2002)
Indonesia	Ehara and Lee (1971)
Japan (Okinawa Is.)	Ehara (1966)
Java	Gutierrez et al (1979)
Korea	Lee et al (1999)
Malaysia (Peninsula)	Rajaratnam and Hock (1975)
Papua New Guinea	Schicha and Gutierrez (1985)
Philippines	McGregor (1950)
Surinam	Flechtman and Etienne (2001); Bolland et al (1998); Flechtman and Knihinicki (2002).
Thailand	Masaki (2001)
Vietnam	Masaki (2001)

Biology

Flechtmann and Knihinicki (2002) assigned the 121 species in the genus *Tetranychus* into nine groups based on female morphology. Group 9, the largest group (38 species), includes *T. piercei*. Six species in the group occur in Australia. Four species in the group, including *T. piercei,* are considered by Flechtmann and Knihinicki (2002) to be of quarantine significance to Australia.

Gutierrez et al (1979) studied a population of *T. piercei* from Indonesia describing the morphology, karyotype, and reproductive activity. These authors collected *T. piercei* from *Polygala paniculata* and cultivated it on *Phaseolus* (bean). They reported the length of males to be from 0.335 mm–0.360 mm and females from 0.460–0.485 mm. The dorsal cuticle of the female is covered in fine hairs and has longitudinal striae (thin lines or bands). Adult females are reddish-brown while the adult male is pinkish-red (Zhang and Fu 2004).

Newly laid eggs are creamy white. Fertile eggs are laid by unmated as well as mated females. Unmated females produce only male progeny and may lay on average 82 eggs during their first 27 days of life, while mated females produce up to 150 eggs (Gutierrez et al 1979).

Fu et al (2002) cultured *T. piercei* on banana leaves. They conducted experiments at 16 °C, 20 °C, 24 °C, 28 °C, 32 °C and 36 °C and found the developmental time from egg to adult varied from 7.2 days at 32–33.1 days at 16 °C. The developmental threshold temperatures (lowest temperature at which development can take place) of egg, larva, protonymph, deutonymph and preoviposition over a whole generation were 11.2 °C, 9.8 °C, 11.9 °C, 12.5 °C, 13.3 °C and 10.7 °C, respectively. The effective accumulated temperatures were 72.3, 33.6, 22.2, 27.8, 17.7 and 163.6 day-degrees [4] respectively.

Survival rate was the highest (96.1%) at 32 °C; whereas at 36 °C and 16 °C it decreased to 79.1% and 66.4%, respectively. The highest average number of eggs (35.8 eggs per female) was recorded at 28 °C; and the lowest at 16 °C (17.9 eggs per female). Female longevity was the longest (34.5 days) at 20 °C and the shortest (8.2 days) at 36 °C. The net reproductive rate was the highest (23.7) at 28 °C

[4] One day-degree is the amount of development that occurs in 24 hours when the temperature is one degree above the development threshold.

and the lowest (5.1) at 36 °C. The intrinsic rate of increase and finite rate of increase reached maximal values (0.35 and 1.42 respectively) at 32 °C, whereas minimal values (0.07 and 1.08 respectively) occurred at 16 °C. The mean generation time and time for population doubling were the shortest at 36 °C and 32 °C respectively, that is, 7.2 days and 8 days respectively. The highest population trend index (25.28) was obtained at 28 °C and the lowest (4.88) at 36 °C.

These results suggest that *T. piercei* could develop and reproduce within a wide range of temperatures, and that temperatures from 26–32 °C are the most suitable conditions for the development, survival and reproduction of the mite.

Tetranychus piercei can be common on *Carica papaya* in Guangdong, People's Republic of China, and Liu and Liu (1986) studied its biology on this host (Hao et al 1996). Peak abundance was from late May to early June. These authors found similar development times to those observed by Fu et al (2002). The egg, larval, nymphal and pre-oviposition stages lasted 3.3–3.8 days, 1.3–1.6 days, 2.9–3.0 days and 1.1–2.0 days respectively, at 28–32 °C and 74–85% relative humidity.

Nymphs and adults spin webs on leaves and stems, with infestation usually starting at the lower parts of the plant and then spreading upwards. Although spider mites are usually found on leaves, they are able to move to other parts of the plants including fruit, especially when populations are high.

Mobile stages suck the juice from tender leaves and stems. In Indonesia in November 1976, *T. piercei* was collected on *Polygala paniculata,* where it prefers the underside of leaves (Gutierrez et al 1979). It infests mature leaves of bananas, causing small brown spots initially on the undersurface. As populations increase, damage spots increase in size and finally the whole undersurface of the leaf becomes reddish-brown. The upper leaf surface also turns yellow and then the entire leaf becomes necrotic and dry. Large infestations cause arrested plant growth, loss of yield, poor quality fruit and delayed harvest times (Fu et al 2002).

Tetranychus piercei is highly polyphagous and its host list is probably larger than that already recorded (Gutierrez et al 1979). In Taiwan, Chen et al (1997) found it was most abundant on flowers and ornamental plants rather than vegetables, dryland crops and fruit trees. It was very common on betel nut and has been intercepted on plants imported to Japan from Korea (Masaki 2001). The species is economically important in Malaysia, where it feeds on young oil palms, particularly if they are deficient in boron (Gutierrez et al 1979). Highest mite densities occurred on seedlings that had received little or no boron in the growing medium. Mite injury was greatest when leaves had the highest mite density and the lowest concentrations of boron and cyanidin (Rajaratnam and Hock 1975). As boron is necessary for proanthocyanidin production in the crop, it was considered likely that the relative concentration of proanthocyanidin in the leaf determines the level of resistance (Rajaratnam and Hock 1975).

Flechtmann and Knihinicki (2002) note that spider mites disperse and exploit new feeding sites very quickly, thereby inflicting severe damage on agricultural and horticultural crops, often leading to economic losses.

In recent years, all of the banana plantations in Hainan province, People's Republic of China, have been attacked by the banana leaf mite, and mite populations can reach high numbers, forcing farmers to apply acaricide several times a year (Fu et al 2002). These heavy acaricide applications tend to exacerbate the problem because natural predators are also killed and resistance develops rapidly. In this region, the mites can go through 26 generations a year and populations are highest during drought or the dry season between October and April. Spraying a 4000 × dilution solution of 1.8% abamectin or a 1500–2000 × dilution solution of 50% Acarol could provide good control at these times (Zhang and Fu 2004). The coccinellid beetle, *Stethorus siphonulus*, was the main natural enemy in China, consuming 16.7 mites or more than 28 eggs daily (Liu and Liu 1986).

Tetranychus piercei has been intercepted by US quarantine officers on *Dracaena* plants (Welbourn 2005). It was noted that the species could become a serious pest if it established in North America.

In a recent trial of the fumigant Vapormate on pests of bananas carried out in the Philippines, mites that were abundant in banana and adjacent papaya plantations were used (Krishna et al 2005).

Specimens in photographs of the trial species have been identified as Tetranychidae, possibly *Tetranychus* sp. (F Beaulieu, Research Scientist, Agriculture and Agri–Food Canada, pers comm 20 June 2006).

13.5 *Tetranychus marianae*

Scientific name

Tetranychus marianae McGregor 1950 [Acari: Tetranychidae]

Synonym

None

Common name

Tropical red spider mite

Hosts

The following host list was taken from Bolland et al (1998).

Abelmoschus esculentus (Okra); *Alocasia macrorhiza* (Elephant Ears); *Alocasia sp.; Arachis hypogaea* (Peanut); *Argyreia sp.*; *Artemisia douglasiana* (Mugwort); *Asystasia gangetica* (Chinese Violet); *Brassica oleracea* (Wild Mustard); *Brugmansia candida* (White Angel's Trumpet); *Buettneria aculeata; Capsicum annuum* (Capsicum); *Carica papaya* (Papaya); *Carica pubescens* (Mountain Papaya); *Centrosema pubescens* (Centro); *Chenopodium ambrosioides* (Epazote); *Colocasia esculenta* (Taro); *Croton sp* (Croton); *Cucumis sativus* (Cucumber); *Cucurbita maxima* (Buttercup Squash); *Cucurbita sp.*; *Datura arborea* (Angel’s Trumpet); *Dieffenbachia picta* (Dumb Cane); *Dioscorea sp* (Yam); *Erythrina sp* (Coral Tree); *Euphorbia hirta* (Asthma Weed); *Gliricidia sepium* (Gliricidia); *Glycine max* (Soya Bean); *Gossypium barbadense* (Pima Cotton); *Gossypium hirsutum* (Upland Cotton); *Gossypium sp* (Cotton); *Guilielma utilis* (Palm); *Hibiscus sp.* (Hibiscus); *Ipomoea aquatica* (Water Spinach); *Ipomoea batatas* (); *Ipomoea sp.* (Sweet Potato); *Ipomoea triloba* (Littlebell); *Lablab purpureus* (Hyacinth Bean); *Lavandula vera* (English Lavender); *Lycopersicon esculentum* (Tomato); *Macroptilium atropurpureum* (Purple Bean); *Malocchia lupulina*; *Manihot esculenta* (Cassava); *Melanolepis multiglandulosa* (Alim); *Merremia vitifolia* (Grape Leaf Morning Glory); *Morus sp.*(Mulberry); *Musa sapientum* (Apple Banana); *Musa sp.* (Banana); *Neonotonia wightii* (Glycine); *Nicotiana glauca* (Mustard Tree); Orchidaceae; *Passiflora biflora* (Passionflower); *Passiflora edulis* (yellow passionfruit); *Passiflora foetida* (Stinking Passion Flower); *Passiflora sp.* (Passionfruit); *Pelargonium sp* (Geranium); *Petiveria alliacea* (Anamu); *Phacelia sp.*; *Phaseolus vulgaris* (Common Bean); *Physalis sp.*; *Piper sp.; Pueraria phaseoloides* (Tropical Kuzdo); *Ricinus communis* (Castor Oil Plant); *Rosa sp.* (Rose); *Salpichroa origanifolia* (Pampas Lily-of-the-Valley); *Sechium edule* (Choko); *Sida acuta* (Common Wireweed); *Sida rhombifolia* (Arrowleaf Sida); *Solanum elaeagnifolium* (Silverleaf Nightshade); *Solanum inducum; Solanum mauritianum* (Woolly Nightshade)*; Solanum melongena* (Eggplant); *Solanum nigrum* (Black Nightshade)*; Solanum sp.* (Nightshade); *Thunbergia alata* (Black-Eyed Susan Vine); *Thunbergia sp.* (Clock Vine); *Triumfetta semitriloba* (Sacramento Bur); *Urena lobata* (Caesar's weed); *Vigna sp*. (Beans); *Wedelia sp.; Zea mays (Corn).*

Plant parts affected

Tetranychus marianae is a cosmopolitan species of tropical and subtropical areas, infesting a wide variety of agricultural plants (Flechtmann and Knihinicki 2002). Colonies of this species are found on the underside of leaves (Ochoa cited in Noronha 2006).

Distribution

Tetranychus marianae is a cosmopolitan species of tropical and subtropical areas (Flechtmann and Knihinicki 2002). Table 13.5 lists the countries where *T. marianae* has been recorded.

Table 13.5 Distribution list for *Tetranychus marianae*

Country	Reference
American Samoa	Bolland et al 1998
Argentina	Bolland et al 1998
Australia (NT)	Bolland et al 1998, APPD 2008
Bahamas	Bolland et al 1998
Brazil	Bolland et al 1998, Noronha 2006
Colombia	Bolland et al 1998
Costa Rica	Bolland et al 1998
Cuba	Bolland et al 1998
Fiji	Bolland et al 1998
French West Indies	Bolland et al 1998
Guam Island	Bolland et al 1998
Honduras	Bolland et al 1998
Mariana Islands	Bolland et al 1998
Marshall Islands	Bolland et al 1998
Mexico	Bolland et al 1998
New Caledonia	Bolland et al 1998
Nicaragua	Bolland et al 1998
Papua New guinea	Bolland et al 1998
Philippines	Bolland et al 1998
Puerto Rico	Bolland et al 1998
Solomon Islands	Bolland et al 1998
Thailand	Bolland et al 1998
USA	Bolland et al 1998
Vanuatu	Bolland et al 1998
Vietnam	Bolland et al 1998
West Indies	Bolland et al 1998
Western Samoa	Bolland et al 1998

Biology

Although little is known about the biology of *T. marianae*, Noronha (2006) conducted preliminary laboratory studies to determine biological aspects of this species. Under laboratory conditions between 24-26°C and 80% RH, the mean incubation period for eggs was 4.63 days and the time from egg to adult was 10.73 days. The mean oviposition period was 19.85 days with each female laying an average of 3.69 eggs/day and reaching the maximum daily oviposition rate as 14 day old adults. The sex ratio of females to males was about 4.3:1 and mean longevity was 24.53 days for females and 8.14 days for males. The lifecycle was completed in about 23 days.

Appendix 14. Weevils

Five species of weevil are of potential quarantine concern to the whole of Australia and occur on bananas in the Philippines.

Scientific names

Philicoptus demissus	Heller 1929 [Coleoptera: Curculionidae]
Philicoptus iliganus	Heller 1929 [Coleoptera: Curculionidae]
Philicoptus sp.1	CN3 in Stephens 1984 [Coleoptera: Curculionidae]
Philicoptus sp.2	CN9 Digos variant and CN10 Dizon variant in Stephens 1984 [Coleoptera: Curculionidae]
Philicoptus strigifrons	Heller 1929 [Coleoptera: Curculionidae]

Synonyms

Philicoptus demissus, P. iliganus and *P. strigifrons* are all from Mindanao and were originally described in the genus *Coptorrhynchus* by Heller (1929). *Philicoptus iliganus* was previously misidentified as *P. waltoni* (Boheman).

Common name

Peel-scarring weevil

Hosts

The following host lists for *P. demissus* and *P. iliganus* were taken from Stephens (1984), Szinicz (2005) and the BPI (2001):

Philicoptus demissus: *Ageratum conyzoides* (chickweed); *Brassica rapa* ssp. *chinensis* (bokchoy/pechay); *Coffea arabica* (coffee); *Crassocephalum crepidioides* (thickweed); *Durio zibethinus* (durian); *Musa* spp. (banana); *Nephelium lappaceus* (rambutan); *Persea americana* (avocado) and *Theobroma cacao* (cacao).

Philicoptus iliganus: *Artocarpus integrifola* (jackfruit); *Citrus* sp.; *Coffea* spp. (coffee); *Dureo zibethinus* (durian); *Garcinia mangostana* (mangosteen); *Glicidia sepium* (madre de cacao); *Lansium domesticum* (lansones); *Musa* spp. (banana); *Nephelium lappaceus* (rambutan); *Persea americana* (avocado); *Phaseolus aureus* (mungbeans); and *Theobroma cacao* (cacao).

Philicoptus sp.1: In laboratory experiments it fed on *Musa sapientum* (banana) and *Gynura cripedioedes* (gapang apoy) (Stephens 1984).

Philicoptus sp.2: *Coffea* spp. **(**coffee). Adults are able to feed on young banana fingers in vitro (Stephens 1984).

Philicoptus strigifrons: Stephens (1984) states that 'the insect is not a proven banana peel-feeding weevil in the field but is suspect since it feeds on banana flowers and fed voraciously on young banana fingers in a 1500 ml beaker.'

Plant Parts Affected

Banana leaves, flower bracts and fruit peel; in heavy infestations, feeding is common on leaf vein ridges (Stephens 1984).

Distribution

All five species are only known from the island of Mindanao and in the absence of any information to the contrary, are assumed to be restricted to tropical climates. They appear to be allopatric (that is, they have separate and mutually exclusive areas of geographical distribution) (Stephens 1984):

- *Philicoptus demissus* is found in the arid General Santos banana zone.
- *Philicoptus iliganus* is distributed west of the Davao River Valley ('south banana zone').
- *Philicoptus* sp. 1 is restricted to the 'north banana zone', north and east of the Lasang River.
- *Philicoptus* sp. 2 is found in the 'north banana zone', north of Digos near Santa Cruz and near Mawab.
- *Philicoptus strigifrons* is found at altitudes of 700 m, between Guinga and the Lapidas River.

Biology

Of the five species, *P. iliganus* is the most severe pest (Stephens 1984). Therefore, there is more information on its biology than for the other four species. The following biological data relate mainly to *P. iliganus*. However, this is likely to be similar for the other species within the genus.

Weevils are members of the beetle order Coleoptera and have four developmental stages: egg, larva, pupa and adult (Lawrence and Britton 1994). The adults of *P. iliganus* range in length from 7–8 mm for females and 6–7 mm for males (Stephens 1984). *Philicoptus* sp. 1, the second most important weevil species as far as damage is concerned, is slightly smaller, with females being 7 mm in length and males 6 mm in length (Stephens 1984). Adult females lay eggs singly or in a mass in the soil and the eggs hatch in 10 days (BPI 2001). In laboratory studies, the egg stage of *Philicoptus* sp.1 lasted 6–10 days, the larval stage 104–165 days, the pupal stage 42–58 days, and the adult stage 33–128 days (Stephens 1984). The total larval period for *P. iliganus* ranges from 102–174 days on banana suckers (BPI 2001). Larvae pupate in a chamber in the soil and this pupal period lasts 10–23 days (BPI 2001). The whole lifecycle from eggs to adults ranges from 111–176 days (BPI 2001).

Adults do not have an effective means of dispersal since they have no hind wings and the elytra (first pair of wings) are firmly united at the suture. They are relatively immobile, unable to move long distances and cannot fly (BPI 2001). When disturbed, they fall to the ground and feign death, thus minimising opportunities to be carried inadvertently with fruit or leaf materials.

In bananas, adults hide in leaf axils, between touching leaves and concealed among fruit (Stephens 1984). During the day, there are periods of inactivity interspersed with periods of active crawling. The destructive stages of the lifecycle are the adult weevil and the larva. Adult weevils feed on the surface of young fingers, causing scarring of the banana peel, while the larvae are thought to feed on the corm or roots of bananas in the soil (Stephens 1984). Adults feed near the base of the youngest leaf of banana plants which have not yet fruited and pronounced feeding can occur on leaf veins. Feeding also occurs on lower bracts before young banana fingers are exposed. When the bracts open, adults enter the flower bud and scar the young fingers. Feeding occurs mostly in young fruit and adults move out of the bunch when the fruit gets older (BPI 2001). Adults tend to feed along fruit ridges and scarring occurs up to harvest time. Damage appears as deep scars on the fruit peel and these scars crack with age (Stephens 1984).

In the Philippines, weevils tend to be found in discrete populations, in that isolated populations are likely to remain within the same area for several years without spreading (Stephens 1984). However, studies by Szinicz (2005) on the island of Leyte in the Philippines show that an unidentified *Philicoptus* species was feeding in vegetable plots of pechay (*Brassica rapa* var. *chinensis*) at distances between 10–27 m from the forest edge. As the habitat of these weevils is the forest, this indicates that they are able to disperse up to 30 m, even though they lack the ability to fly.

Appendix 15. Thrips

Two species of thrips are of quarantine significance to Western Australia and occur on bananas in the Philippines.

Scientific Names

Chaetanaphothrips signipennis Bagnall 1913 [Thysanoptera: Thripidae]
Elixothrips brevisetis (Bagnall 1919) [Thysanoptera: Thripidae]

Synonyms

Chaetanaphothrips (Scirtothrips) signipennis Bagnall
Scirtothrips signipennis Bagnall
Anaphothrips signipennis
Chaetanaphothrips biguttaticorpus
Euthrips biguttaticorpus Girault 1924
Physothrips citricorpus Girault 1927
There are no synonyms for *E. brevisetis*

Common Names

Chaetanaphothrips signipennis: Banana rust thrips, red rust thrips.
Elixothrips brevisetis: Banana rust thrips, banana rind thrips.

Hosts

Chaetanaphothrips signipennis: *Anthurium* sp.; *Citrus reticulata* (tangerine/mandarin); *Cordyline fruticosa* (ti, good-luck-plant); *Dracaena* sp.; *Musa* sp. (banana); *Musa x paradisiaca* (plantain); *Maranta leuconeura* (banded arrowroot); *Strelitzia reginae* (Queens bird-of-paradise) (Denmark and Osborne 1985). The species also infests the immature fruits of *Citrus sinensis* (orange), *Lycopersicon esculentum* (tomatoes) and *Phaseolus vulgaris* (green beans) (Hara et al 2002).

Elixothrips brevisetis: *Canna* spp.; *Carica papaya* (papaya); *Cestrum pallidum*; *Dioscorea* spp.; *Ficus* spp.; *Ipomea alba*; *Musa* sp. (banana); *Morinda citrifolia*; *Wedelia trilobata* (Mau and Kessing 1993a).

Plant Parts Affected

Fruit, leaves, pseudostem (Trevorrow 2002; Hara et al 2002).

Distribution

Table 15.1 and Table 15.2 list countries where *C. signipennis* and *E. brevisetis* have been recorded.

Table 15.1 Distribution list for Chaetanaphothrips signipennis

Country	References
Australia (New South Wales and Queensland)	Trevorrow (2002)
Brazil	Denmark and Osborne (1985); Hara et al (2002)
Fiji	Denmark and Osborne (1985); Hara et al (2002)
Honduras	Denmark and Osborne (1985); Hara et al (2002)
India	Denmark and Osborne (1985); Hara et al (2002)
Panama	Denmark and Osborne (1985); Hara et al (2002)
Philippines	PCARRD (1998)

Country	References
Sri Lanka	Denmark and Osborne (1985); Hara et al (2002)
USA (Florida, Hawaii)	Denmark and Osborne (1985); Hara et al (2002)

Table 15.2 Distribution list for *Elixothrips brevisetis*

Country	References
Australia (Northern Territory and Queensland)	NT, Qld (APPD 2005; Mound 2002)
Guam	Mau and Kessing 1993a
Marianas archipelago	Mau and Kessing 1993a
Philippines	Mau and Kessing 1993a
Republic of Seychelles	Mau and Kessing 1993a
Taiwan	Mau and Kessing 1993a
USA (Hawaii)	Mau and Kessing 1993a

Biology

There is no published information on the biology of *E. brevisetis.*

The life stages of banana rust thrips *C. signipennis* (Bagnall) include adult, egg, larva (nymph), pre-pupa and pupa.

The adults are creamy yellow to golden brown and 1–1.6 mm long (Hara et al 2002). The wings are fringed with dark eye-like spots at the base; when the wings are folded, the adult appears to have a black line down its back (Pinese and Piper 1994).

Banana rust thrips reproduce sexually. After mating, females lay kidney-shaped eggs in the plant tissue, just below the surface of the fruit or under leaf sheaths (Pinese and Piper 1994; Hara et al 2002; Trevorrow 2002). These eggs hatch after about a week. The newly hatched larvae are white to cream and, when fully developed after about another week, are about the same size and shape as the adults, but have no wings (Trevorrow 2002). The mature larvae enter the soil and develop into pre-pupae, and then form white pupae that look like mature larvae. Adult thrips emerge from the pupae 7–10 days later.

The lifecycle (from egg to adult) is completed in approximately 28 days, but it may take up to three months during cooler seasons (Hara et al 2002). There are many generations per year, but the greatest numbers of thrips occur from November to March (Trevorrow 2002).

Although adult thrips can fly, major spread is far more likely by movement of infested planting material to new areas (Trevorrow 2002).

Both adults and larvae feed by puncturing plant surface cells and sucking up the sap. Banana rust thrips can cause damage on fruit throughout the year, but the period from November to April is the period of greatest pest activity (Pinese and Piper 1994).

Banana rust thrips may infest bunches at any time during their growth, but infestations that develop when bunches emerge result in the most severe damage (Trevorrow 2002). Injured areas on young fruit first look water soaked, then discoloured and grey and later become rust coloured. With further growth of the fruit, cracks may develop in the scarred areas. Injury to fruit is usually on the sides of fruit that are touching or are close together, but in severe infestations the whole fruit may be blemished. Sometimes the fruits split.

The larvae and adults congregate in colonies on the pseudostems behind the bases of leaf sheaths, and their feeding leaves the plant tissue blood red in colour. They congregate on fruit, mainly where the fruits touch each other (Braithwaite 1963).