Australian Government

Biosecurity Australia

# Draft

# Pest Risk Analysis Report for '*Candidatus* Liberibacter species' and their vectors associated with Rutaceae

## August 2010

Cite this report as:

Plant Biosecurity (2010) Draft pest risk analysis report for '*Candidatus* Liberibacter species' and their vectors associated with Rutaceous hosts. Plant Biosecurity, Canberra.



Postal address:

Biosecurity Australia
GPO Box 858
CANBERRA ACT 2601
AUSTRALIA

Internet: www.biosecurityaustralia.gov.au

Cover images: Symptoms of infection of '*Candidatus* Liberibacter species' in citrus and its vectors *Diaphorina citri* and *Trioza erytreae* http://www.apsnet.org/online/archive/1999/IW00006.jpg

## Submissions

This draft pest risk analysis (PRA) report has been issued to give all interested parties an opportunity to comment and draw attention to any scientific, technical, or other gaps in the data, misinterpretations and errors. Any comments should be submitted to Plant Biosecurity within the comment period stated in the related Biosecurity Australia Advice on the Biosecurity Australia website. The draft PRA report will then be revised as necessary to take account of the comments received and a provisional final PRA report will be released at a later date.

Comments on the draft PRA report should be submitted to:

Plant Biosecurity
Biosecurity Australia
GPO Box 858
CANBERRA ACT 2601
AUSTRALIA

Telephone: +61 2 6272 5094
Facsimile: +61 2 6272 3307
Email: plant@biosecurity.gov.au

Internet: www.biosecurityaustralia.gov.au

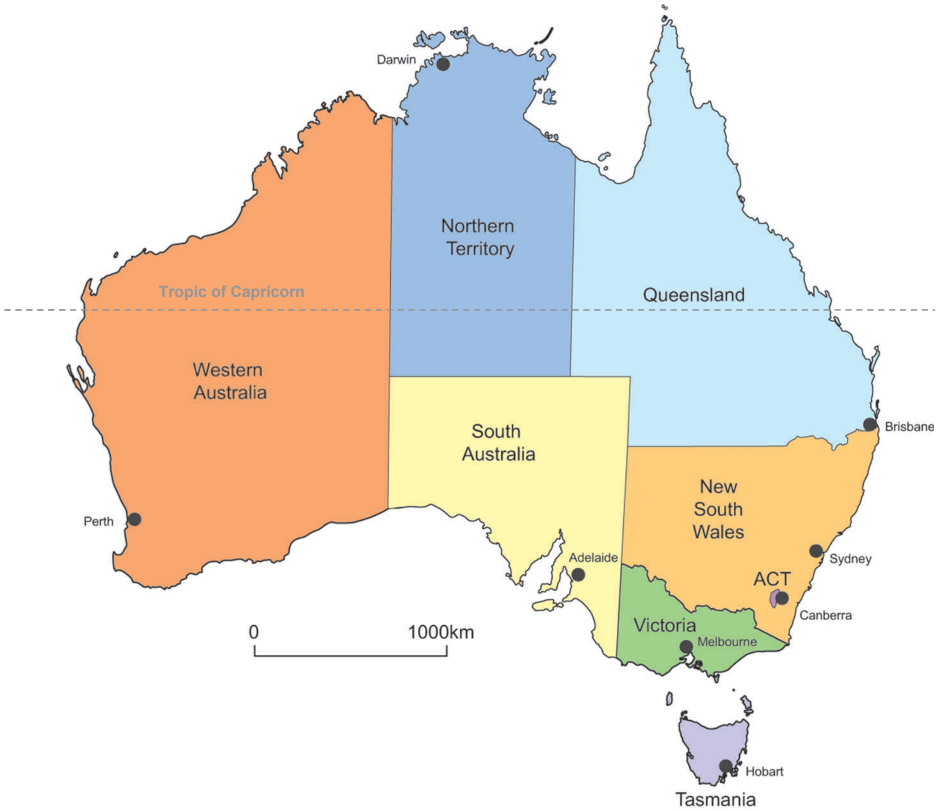
# Contents

## Tables

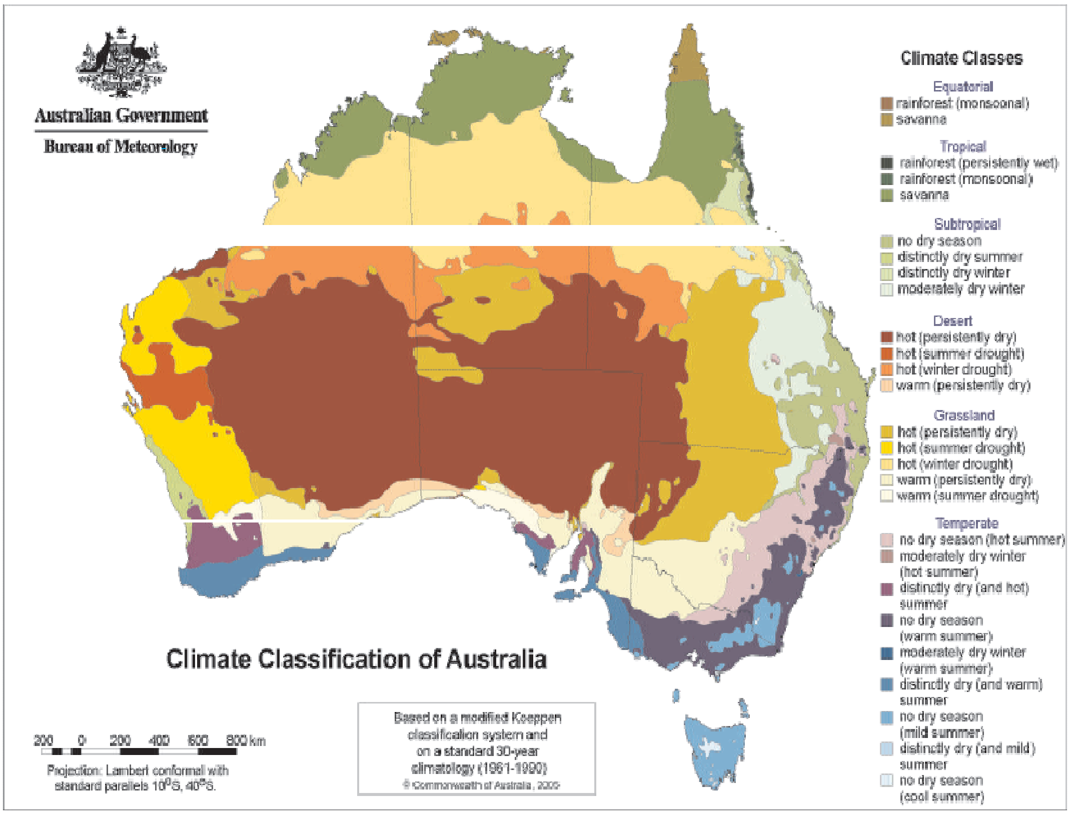


## Figures

## Figure i Map of Australia




## Figure ii A guide to Australia's bio-climate zones



# Acronyms and abbreviations

| Term or abbreviation | Definition |
|---|---|
| **ALOP** | Appropriate level of protection |
| **APPD** | Australian Plant Pest Database (Plant Health Australia) |
| **AQIS** | Australian Quarantine and Inspection Service |
| **CABI** | CAB International, Wallingford, UK |
| **CDFA** | Californian Department of Food and Agriculture |
| **CMI** | Commonwealth Mycological Institute |
| **CSIRO** | Commonwealth Scientific and Industrial Research Organisation |
| **DAFF** | Australian Government Department of Agriculture, Fisheries and Forestry |
| **ELISA** | Enzyme-linked immuno-sorbent assay |
| **FAO** | Food and Agriculture Organization of the United Nations |
| **HLB** | Huanglongbing |
| **IPC** | International Phytosanitary Certificate |
| **IPM** | Integrated Pest Management |
| **IPPC** | International Plant Protection Convention |
| **IRA** | Import Risk Analysis |
| **ISPM** | International Standard for Phytosanitary Measures |
| **NPPO** | National Plant Protection Organization |
| **NSW** | New South Wales |
| **NT** | Northern Territory |
| **PCR** | Polymerase chain reaction |
| **PEQ** | Post-entry quarantine |
| **PRA** | Pest risk analysis |
| **Qld.** | Queensland |
| **SA** | South Australia |
| **SPS** | Sanitary and phytosanitary |
| **Tas.** | Tasmania |
| **USDA** | United States Department of Agriculture |
| **Vic.** | Victoria |
| **WA** | Western Australia |
| **WTO** | World Trade Organisation |

# Abbreviations of units

| Term or abbreviation | Definition |
|---|---|
| **ºC** | degree Celsius |
| **ºF** | degree Fahrenheit |
| **RH** | relative humidity (expressed as a percentage) |
| **km** | kilometre |
| **$LT_{50}$s** | time taken for 50% mortality of a population |
| **m** | metre |
| **mm** | millimetre |

# Summary

Plant Biosecurity has undertaken this qualitative risk analysis to assess the quarantine risks posed by '*Candidatus* Liberibacter africanus', '*Ca.* L. americanus' and '*Ca.* L. asiaticus', and their citrus psyllid vectors, *Diaphorina citri* and *Trioza erytreae*.

The pest risk analysis has identified nursery stock (live plants), budwood, seed for sowing and psyllid vectors infected with '*Ca*. L. species' as potential pathways for the introduction of '*Ca*. L. species' into Australia. Fruit, nursery stock, budwood and fresh leaves were identified as potential pathways for the introduction of *D. citri* and *T. erytreae*. These potential pathways have an unrestricted risk that exceeds Australia's appropriate level of protection (ALOP).

Australia's existing policy to import citrus budwood and seed for sowing is based on tiered safeguards. That is, if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed. The existing policy is designed to obtain a suitable reduction in risk and to apply additional safeguards as required. Currently, live plants of citrus and other allied genera in the Rutaceae family are not allowed entry into Australia. This draft pest risk analysis report supports the existing policy. This draft pest risk analysis report recommends that additional measures be applied to budwood and seed for sowing of all known hosts of '*Ca*. L. species'. Specifically, the proposed measures are:

**For budwood (potentially carrying '*Ca.* L. species' and citrus psyllids):**

- extending the existing policy for citrus budwood to all hosts of '*Ca*. L. species' (i.e., on arrival inspection, mandatory fumigation and mandatory growth in closed government post-entry quarantine (PEQ) facilities until the required pathogen screening/testing is completed); and
- additionally, plants grafted with imported budwood must be grown at 22–24 °C to promote symptom expression for '*Ca.* L. species' and polymerase chain reaction (PCR) testing for '*Ca.* L. species' must be undertaken prior to release.

**For seed for sowing (potentially carrying '*Ca.* L. species'):**

- hot water treatment of seed at 50 °C for 20 minutes from all sources; or
- if seed is sensitive to heat treatment, mandatory surface sterilization and growth at 22–24 °C in closed government PEQ facilities for 24 months with pathogen screening including PCR testing for '*Ca*. L. species' prior to release.

This draft pest risk analysis report recommends that risk management measures are required to import citrus fruit from areas where psyllid vectors are known to occur. Specifically, the proposed measures are:

**For fruit (potentially carrying citrus psyllids):**

- area freedom from citrus psyllids; or
- a systems approach using pre- and post-harvest measures to ensure that fruit is not infested with psyllids; or
- an application of a treatment to fruit known to be effective against all life stages of the psyllid (including but not limited to methyl bromide fumigation).

This draft pest risk analysis report recommends that risk management measures are required to imported fresh leaves of all known hosts of *D. citri* and *T. erytreae* where psyllid vectors are known to occur. Specifically, the proposed measures are:

**For fresh leaves (potentially carrying citrus psyllids):**

- area freedom; or
- hot air treatment at 85 °C for 8 hours; or
- an application of a treatment to fresh leaves known to be effective against all life stages of the psyllid (including but not limited to methyl bromide fumigation).

Interested parties can provide comments and submissions to Plant Biosecurity within the consultation period. Plant Biosecurity will consider any comments received before finalising the pest risk analysis and quarantine policy recommendations.

# 1 Introduction

## 1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests[1] entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The pest risk analysis (PRA) process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the risks that could be associated with proposals to import products into Australia. If the risks are found to exceed Australia's appropriate level of protection (ALOP), risk management measures are proposed to reduce the risks to an acceptable level. But if it is not possible to reduce the risks to an acceptable level, then no trade will be allowed.

Successive Australian governments have maintained a conservative, but not a zero-risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's PRAs are undertaken by Plant Biosecurity using teams of technical and scientific experts in relevant fields, and involves consultation with stakeholders at various stages during the process. Plant Biosecurity provides recommendations for animal and plant quarantine policy to Australia's Director of Animal and Plant Quarantine (the Secretary of the Australian Department of Agriculture, Fisheries and Forestry). The Director or delegate is responsible for determining whether or not an importation can be permitted under the *Quarantine Act 1908*, and if so, under what conditions. The Australian Quarantine and Inspection Service (AQIS) is responsible for implementing appropriate risk management measures.

More information about Australia's biosecurity framework is provided in the *Import Risk Analysis Handbook 2007* (update 2009) located on the Biosecurity Australia website www.biosecurityaustralia.gov.au

## 1.2 This pest risk analysis

### 1.2.1 Background

'*Candidatus* Liberibacter species' cause Huanglongbing (HLB) disease in citrus and are considered one of the most destructive diseases of citrus worldwide (Zhang *et al*. 2010). The pathogen and its vectors occur on all species and cultivars of citrus and on some non-citrus members of the Rutaceae (Van den Berg 1990; Halbert and Manjunath 2004; da Graça 2008). Australia is currently free from '*Ca.* L. species' and their known vectors, *Diaphorina citri* and *Trioza erytreae*.

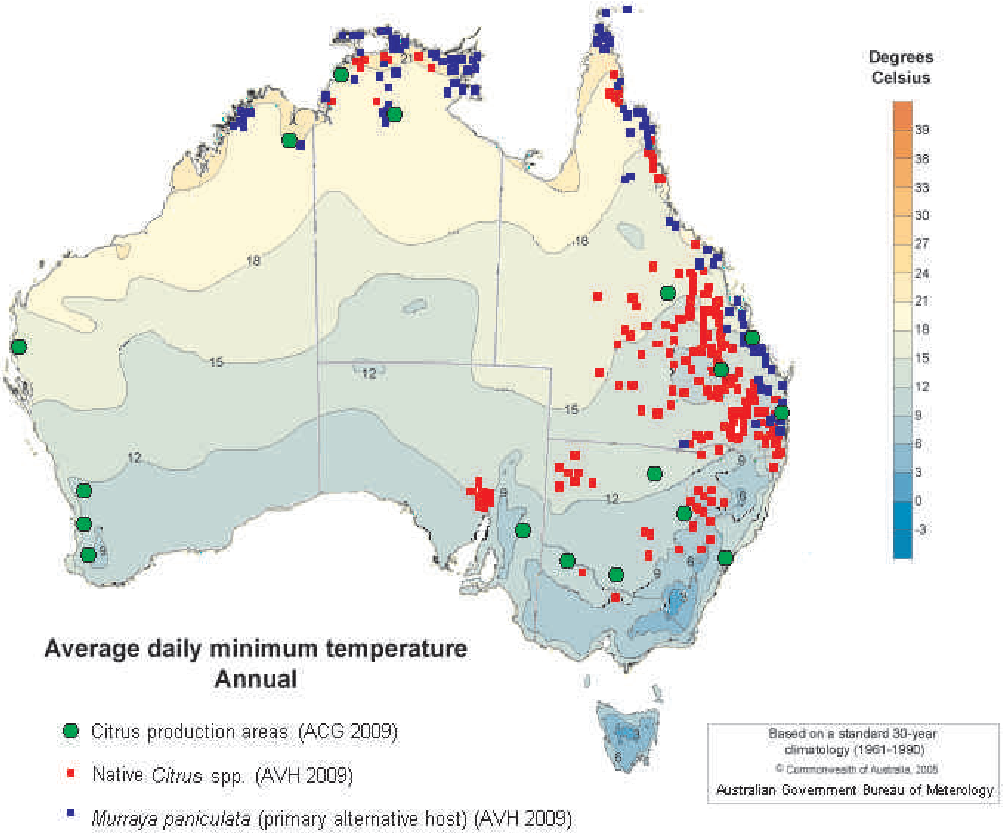
Currently, imports of fresh citrus fruit are only permitted from Egypt, Israel, Italy, New Zealand, Spain and the USA (Arizona, California and Texas). In recent years, '*Ca.* L.

[1] A pest is any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (FAO 2007).

species' and their vectors have extended their range around the world. The continued expansion in range of '*Ca.* L. species' and their vectors, particularly towards countries and regions that are approved to export fresh fruit to Australia, and the identification of new pathways for their entry has resulted in a renewed interest in these pathogens. Plant Biosecurity initiated this PRA to assess the quarantine risks associated with these pests and the importation of host plant material into Australia.

The wide distribution of suitable hosts—including the common garden plant and primary alternative host, *Murraya paniculata*—for the establishment of '*Ca.* L. species' and their vectors raised quarantine concerns about these important pests for Australia. Furthermore, the suitable temperate climate of Australia, particularly the mild winter temperatures, will assist in the establishment of these pests if they enter Australia (Figure 1.1).

**Figure 1.1: Distribution of suitable hosts of '*Ca.* L. species', their vectors and mean annual minimum temperature in Australia**




In a number of instances the HLB bacterium has been detected not long after the vector has established in a region. For example, *D. citri* was first reported in Florida in 1998 and the HLB bacterium (Asian strain) was reported in 2005; the psyllid was reported in Cuba in 2001 and Mexico 2002 (Halbert and Núñez 2004) and the bacterium (Asian strain) reported in 2008 in Cuba (Llauger *et* al. 2008) and 2009 in Mexico (NAPPO 2009a). In contrast, *D. citri* was reported in Brazil in 1942 and the HLB bacteria (Asian and American species) were not reported until 2004 (Bové 2006). Plants infected with HLB bacteria may be asymptomatic in the initial stages of infection and can be easily overlooked. Additionally, symptoms caused by HLB bacteria are influenced by temperature. In Brazil and Florida, HLB was present and unrecognized for several years (~5–10 years) before it was reported. When eventually reported, the disease was so widespread that its eradication had become unfeasible (Bové 2006).

Nursery stock has been considered the main means of long distance spread of '*Ca.* L. species' and their vectors (Bové 2006). In 2004, fresh citrus fruit was confirmed as a pathway for *D. citri* (Halbert and Núñez 2004). In 2008, '*Ca.* L. species' were confirmed to be present in fresh citrus fruit and on citrus seed coats but not in the seed endosperm and embryo (Tatineni *et al* 2008).

### 1.2.2 Scope

In this PRA the risk of importation of three '*Ca.* L. species' are assessed separately. This is due to significant differences in their climatic tolerances, geographic range and psyllid vectors.

This PRA assesses the biosecurity risks of the importation of '*Ca.* L. species' in the following pathways:

- fruits of citrus species permitted entry into Australia.
- nursery stock (live plants) of Rutaceous plants permitted entry into Australia.
- budwood of *Citrus* species permitted entry into Australia.
- fresh leaves of Rutaceous plants permitted entry into Australia.
- seed for sowing of Rutaceous plants permitted entry into Australia.
- infected citrus psyllids (*D. citri* and *T. erytreae*).

and

the biosecurity risks of the importation of *Diaphorina citri* and *Trioza erytreae* in the following pathways:

- fruits of citrus species permitted entry into Australia.
- nursery stock (live plants) of Rutaceous plants permitted entry into Australia.
- budwood of *Citrus* species permitted entry into Australia.
- fresh leaves of Rutaceous plants permitted entry into Australia.

The risk for these pathways was estimated using information on the biology, epidemiology and impact of '*Ca.* L. species' pathogenic to the Rutaceae and their vectors *Diaphorina citri* and *Trioza erytreae*.

Phytosanitary conditions exist for the importation of fruit for consumption, seed for sowing and nursery stock of *Citrus* species into Australia. These conditions include pre-clearance or on-arrival inspection by AQIS of 600 citrus fruit from specified countries, mandatory sodium hypochlorite treatment and hot water treatment for seed for sowing and mandatory fumigation and growth in post-entry quarantine (PEQ) at a government station for nursery stock of *Citrus* species.

However, this pest risk analysis does not consider these specific phytosanitary measures during the pest risk assessment for these pathways. Phytosanitary measures already in place are considered during the development of risk management measures, if they are required, following the pest risk assessment.

The PRA proposes measures that could be used to reduce the risk of the importation of '*Ca.* L. species' pathogenic to the Rutaceae to meet Australia's ALOP. These measures will form the basis for any recommended amendments to the import policy for commodities that are, or were, permitted access to Australia, and are hosts of '*Ca.* L. species' pathogenic to the Rutaceae and its known vectors.

### 1.2.3 Existing policy

**Fresh citrus fruit**

Fresh citrus fruit may be imported into Australia for human consumption from Egypt, Israel, Italy, New Zealand, Spain and the USA (Arizona, California, Texas), subject to specific import conditions. Details of the current import requirements for citrus fruit are available from the AQIS Import Conditions database (ICON) http://www.aqis.gov.au/icon. Currently, imports of fresh citrus fruit are only permitted from countries, or areas, free of '*Ca.* L. species' and its vectors.

The general requirements (Condition C6000) include an AQIS import permit, a quarantine entry, a phytosanitary certificate, freedom from regulated articles and on-arrival inspection and remedial action by AQIS for all countries. In addition to such general measures, specific quarantine/biosecurity measures for each of these countries have also been developed (Table 1.1).

**Table 1.1: Specific quarantine/biosecurity measures for fresh citrus fruit**

| Country | ICON Condition | Condition title |
|---|---|---|
| **Egypt** | C9488 | Fresh citrus from Egypt |
| | C9502 | Fresh fruit species |
| **Israel** | C6027 | Fresh citrus from Israel |
| **Italy** | C10464 | Fresh citrus from Italy |
| **Spain** | C6061 | Pre-shipment or in-transit cold treatment for the disinfestation of Medfly |
| | C6062 | In-transit Cold Treatment – Verification on arrival |
| **USA** | C6109 | Fresh citrus from Arizona, California and Texas only |
| | C6026 | USA citrus: pre-cleared or on-arrival inspection |

***Citrus* species nursery stock (live plants)**

Currently, live plants of citrus are not permitted entry into Australia.

***Citrus* species budwood**

Currently, citrus budwood is permitted entry into Australia from all countries subject to specific import conditions (Condition C7319). Details of the current import requirements for citrus budwood are available from ICON and set out below.

All imported consignments of citrus budwood are subject to the quarantine/biosecurity measures set out in Condition C7300 'General Import requirements, nursery stock for all species'. The general requirements (Condition C7300) include:

- an AQIS import permit
- freedom from regulated articles
- on-arrival inspection

In addition to such general measures, specific quarantine/biosecurity measures have also been developed.

All imported consignments of citrus budwood for propagation are subject to quarantine/biosecurity measures including an AQIS import permit, a phytosanitary certificate, freedom from regulated articles and on-arrival inspection and remedial action by AQIS. In addition to such general measures, the following specific quarantine/biosecurity measures have also been developed.

- All plant material must be fumigated with methyl bromide (T9060)
- All plant material must be treated by dipping in sodium hypochlorite (T9374)
- Mandatory PEQ growth: plants propagated from imported budwood must be grown in closed quarantine for a minimum of 24 months with disease screening (or until the required disease screening/testing is completed). While growing in quarantine, plants will be subject to disease elimination treatments (shoot-tip grafting), passive screening and active testing (biological indexing using woody indicators, serological testing using Enzyme-linked immuno-sorbent assay (ELISA) and molecular testing using Sequential-PAGE)
- Budwood sourced from AQIS approved sources may be exempt from some of the testing requirements provided it is accompanied by:
  - a phytosanitary certificate, which is endorsed with the details of the virus status and source of budwood
  - an exporter's declaration clearly outlining the procedures adopted to disease screen the material, including details and dates of all indexing carried out, indicators/methods used, where the material has been held since indexing and any other treatments applied
- No material will be released from quarantine until all testing and screening procedures have been completed and the material is found to have no evidence of quarantine pests.

**Leaves (fresh)**

Currently, fresh leaves of *Murraya koenigii* (synonym: *Bergena koenigii*) are permitted entry into Australia from European countries, New Zealand, South Pacific Commission countries and the USA (California), and *Aegle marmelos* are permitted from New Zealand and South Pacific Commission countries, subject to specific import conditions. Details of the current import requirements for fresh leaves are available from ICON and set out below.

General import requirements include an AQIS import permit, a quarantine entry, a phytosanitary certificate, freedom from regulated articles and on-arrival inspection and remedial action by AQIS. In addition to such general measures, specific quarantine/biosecurity measures for each of these countries have also been developed:

Condition C6007: Curry leaves (fresh) — Import conditions for leaves only

Condition C6043: Curry leaves (fresh) — Consignments must be heat treated at 85 °C for 8 hrs

Condition C6106: Curry leaves (fresh) — Entry is only permitted from the State of California in the USA (after the recent detection of Asian citrus psyllids emergency measures were put in place)

***Citrus* species seed for sowing**

Currently, citrus seed for sowing is permitted entry into Australia from South Africa, Spain and the USA, subject to specific import conditions. Details of the current import requirements for citrus seed for sowing are available from ICON and set out below.

All imported consignments of citrus seed for sowing from South Africa, Spain and the USA are generally subject to quarantine/biosecurity measures including an AQIS import permit, a phytosanitary certificate, freedom from regulated articles and on-arrival inspection and remedial action by AQIS. In addition to such general measures, specific quarantine/biosecurity measures for each of these countries have also been developed (Table 1.2).

**Table 1.2: Specific quarantine measures for citrus seed for sowing**

| Country | ICON Condition | Details |
|---|---|---|
| **South Africa** | C17777 | A phytosanitary certificate must accompany every consignment of citrus seed from South Africa and bear the following additional declaration:<br>a) 'This seed was sourced from South Africa where *Xylella fastidiosa* is not known to occur'; and<br>b) 'This seed is free from infection with Citrus greening bacterium ('*Candidatus* Liberibacter africanus'), Mal secco (*Phoma tracheiphila*) and Citrus canker (*Xanthomonas axonopodis* pv. *citri*)'. |
| **Spain** | C17776 | A phytosanitary certificate must accompany every consignment of citrus seed from Spain and bear the following additional declaration:<br>a) 'This seed was sourced from Spain where *Xylella fastidiosa* is not known to occur'; and<br>b) 'This seed is free from infection with Citrus greening bacterium ('*Candidatus* Liberibacter africanus'), Mal secco (*Phoma tracheiphila*) and Citrus canker (*Xanthomonas axonopodis* pv. *citri*)'. |
| **USA** | C17778 | All seed requires mandatory treatment in accordance with one of the following:<br>a) surface sterilised in a solution of 8-hydroxyquinoline sulphate (1g/0.1L) for three minutes (T9321); **or**<br>b) surface sterilised in a solution of 1% sodium hypochlorite (1% available chlorine) for 10 minutes (T9371).<br>In addition to the treatment specified (T9321; T9371), all seed is also subjected to **one** of the following:<br>c) Immersed in hot water at 50 ºC for 20 minutes (T9504); **or**<br>d) Growth in a government PEQ facility for a minimum of two years. |

### *Rutaceous hosts* (other than *Citrus* species) nursery stock (live plants)

Currently, live plants of allied genera in the Rutaceae family are not permitted entry into Australia.

### *Rutaceous hosts* (other than *Citrus* species) seed for sowing

Currently, seed of allied genera in the Rutaceae family for sowing are not permitted entry into Australia.

# 2 Method for pest risk analysis

Plant Biosecurity has conducted this pest risk analysis (PRA) in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: *Framework for Pest Risk Analysis* (FAO 2007) and ISPM 11: *Pest Risk Analysis for Quarantine Pests, including analysis of environmental risks and living modified organisms* (FAO 2004).

A PRA is 'the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it' (FAO 2009). A pest is 'any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products' (FAO 2009).

Quarantine risk consists of two major components: the probability of a pest entering, establishing and spreading in Australia from imports; and the consequences should this happen. These two components are combined to give an overall estimate of the risk.

Unrestricted risk is estimated taking into account the existing commercial production practices of the exporting country and that minimal on-arrival verification procedures will apply. Restricted risk is estimated with phytosanitary measure(s) applied. A phytosanitary measure is 'any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests' (FAO 2009).

A glossary of the terms used is provided at the back of this PRA report.

PRAs are conducted in three consecutive stages.

## 2.1 Stage 1: Initiation

The initiation of the pest risk analysis identifies the pest(s) and pathway(s) that are of quarantine concern and should be considered for risk analysis in relation to the identified PRA area.

For this PRA, the 'PRA area' is defined as all of Australia.

## 2.2 Stage 2: Pest risk assessment

A pest risk assessment (for quarantine pests) is: 'the evaluation of the probability of the introduction and spread of a pest and of the likelihood of associated potential economic consequences' (FAO 2009).

The following three, consecutive steps were used in this pest risk assessment:

### 2.2.1 Pest categorisation

Pest categorisation identifies if the pests identified in Stage 1: Initiation require a pest risk assessment. The categorisation process examines, for each pest, whether the criteria in the definition of a quarantine pest are satisfied. A 'quarantine pest' is a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled, as defined in ISPM 5: *Glossary of phytosanitary terms* (FAO 2009).

The pests identified in Stage 1: Initiation were categorised using the following primary elements:

- presence or absence in the PRA area
- regulatory status
- potential for establishment and spread in the PRA area
- potential for economic consequences (including environmental consequences) in the PRA area.

### 2.2.2 Assessment of the probability of entry, establishment and spread

Details of how to assess the probability of entry, probability of establishment and probability of spread of a pest are given in ISPM 11 (FAO 2004). A summary of this process is given below, followed by a description of the qualitative methodology used in this PRA.

**Probability of entry**

The probability of entry describes the probability that a quarantine pest will enter Australia as a result of trade in a given commodity, be distributed in a viable state in the PRA area and subsequently be transferred to a host. It is based on pathway scenarios depicting necessary steps in the sourcing of the commodity for export, its processing, transport and storage, its use in Australia and the generation and disposal of waste. In particular, the ability of the pest to survive is considered for each of these various stages.

For the purpose of considering the probability of entry, Plant Biosecurity divides this step of this stage of the PRA into two components:

**Probability of importation**: the probability that a pest will arrive in Australia when a given commodity is imported

**Probability of distribution**: the probability that the pest will be distributed, as a result of the processing, sale or disposal of the commodity, in the PRA area and subsequently transfer to a susceptible part of a host.

Factors considered in the probability of importation include:

- distribution and incidence of the pest in the source area
- occurrence of the pest in a life-stage that would be associated with the commodity
- volume and frequency of movement of the commodity along each pathway
- seasonal timing of imports
- pest management, cultural and commercial procedures applied at the place of origin
- speed of transport and conditions of storage compared with the duration of the lifecycle of the pest
- vulnerability of the life-stages of the pest during transport or storage
- incidence of the pest likely to be associated with a consignment

- commercial procedures (e.g. refrigeration) applied to consignments during transport and storage in the country of origin, and during transport to Australia.

Factors considered in the probability of distribution include:

- commercial procedures (e.g. refrigeration) applied to consignments during distribution in Australia
- dispersal mechanisms of the pest, including vectors, to allow movement from the pathway to a host
- whether the imported commodity is to be sent to a few or many destination points in the PRA area
- proximity of entry, transit and destination points to hosts
- time of year at which import takes place
- intended use of the commodity (e.g. for planting, processing or consumption)
- risks from by-products and waste.

**Probability of establishment**

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO 2004). In order to estimate the probability of establishment of a pest, reliable biological information (lifecycle, host range, epidemiology, survival, etc.) is obtained from the areas where the pest currently occurs. The situation in the PRA area can then be compared with that in the areas where it currently occurs and expert judgement used to assess the probability of establishment.

Factors considered in the probability of establishment in the PRA area include:

- availability of hosts, alternative hosts and vectors
- suitability of the environment
- reproductive strategy and potential for adaptation
- minimum population needed for establishment
- cultural practices and control measures.

**Probability of spread**

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO 2004). The probability of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. In order to estimate the probability of spread of the pest, reliable biological information is obtained from areas where the pest currently occurs. The situation in the PRA area is then compared with that in the areas where the pest currently occurs and expert judgement used to assess the probability of spread.

Factors considered in the probability of spread include:

- suitability of the natural and/or managed environment for natural spread of the pest
- presence of natural barriers
- potential for movement with commodities, conveyances or by vectors

- intended use of the commodity
- potential vectors of the pest in the PRA area
- potential natural enemies of the pest in the PRA area.

*Assigning qualitative likelihoods for the probability of entry, establishment and spread*

In its qualitative PRAs, Plant Biosecurity uses the term 'likelihood' for the descriptors it uses for its estimates of probability of entry, establishment and spread. Qualitative likelihoods are assigned to each step of entry, establishment and spread. Six descriptors are used: high; moderate; low; very low; extremely low; and negligible (Table 2.1). Descriptive definitions for these descriptors and their indicative probability ranges are given in Table 2.1. The indicative probability ranges are only provided to illustrate the boundaries of the descriptors. These indicative probability ranges are not used beyond this purpose in qualitative PRAs. The standardised likelihood descriptors and the associated indicative probability ranges provide guidance to the risk analyst and promote consistency between different risk analyses.

**Table 2.1: Nomenclature for qualitative likelihoods**

| Likelihood | Descriptive definition | Indicative probability (P) range |
|---|---|---|
| **High** | The event would be very likely to occur | $0.7 < P \leq 1$ |
| **Moderate** | The event would occur with an even probability | $0.3 < P \leq 0.7$ |
| **Low** | The event would be unlikely to occur | $0.05 < P \leq 0.3$ |
| **Very low** | The event would be very unlikely to occur | $0.001 < P \leq 0.05$ |
| **Extremely low** | The event would be extremely unlikely to occur | $0.000001 < P \leq 0.001$ |
| **Negligible** | The event would almost certainly not occur | $0 \leq P \leq 0.000001$ |

The likelihood of entry is determined by combining the likelihood that the pest will be imported into the PRA area and the likelihood that the pest will be distributed within the PRA area, using a matrix of rules (Table 2.2). This matrix is then used to combine the likelihood of entry and the likelihood of establishment, and the likelihood of entry and establishment is then combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

For example, if the probability of importation is assigned a likelihood of 'low' and the probability of distribution is assigned a likelihood of 'moderate', then they are combined to give a likelihood of 'low' for the probability of entry. The likelihood for the probability of entry is then combined with the likelihood assigned to the probability of establishment (e.g. 'high') to give a likelihood for the probability of entry and establishment of 'low'. The likelihood for the probability of entry and establishment is then combined with the likelihood assigned to the probability of spread (e.g. 'very low') to give the overall likelihood for the probability of entry, establishment and spread of 'very low'.

**Table 2.2: Matrix of rules for combining descriptive likelihoods**

| | High | Moderate | Low | Very low | Extremely low | Negligible |
|---|---|---|---|---|---|---|
| **High** | High | Moderate | Low | Very low | Extremely low | Negligible |
| **Moderate** | | Low | Low | Very low | Extremely low | Negligible |
| **Low** | | | Very low | Very low | Extremely low | Negligible |
| **Very low** | | | | Extremely low | Extremely low | Negligible |
| **Extremely low** | | | | | Negligible | Negligible |
| **Negligible** | | | | | | Negligible |

### 2.2.3 Assessment of potential consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the likely consequences if the pests or disease agents were to enter, establish and spread in Australia. The assessment considers direct and indirect pest effects and their economic and environmental consequences. The requirements for assessing potential consequences are given in Article 5.3 of the SPS Agreement (WTO 1995), ISPM 5 (FAO 2009) and ISPM 11 (FAO 2004).

Direct pest effects are considered in the context of the effects on:

- plant life or health
- other aspects of the environment.

Indirect pest effects are considered in the context of the effects on:

- eradication, control, etc
- domestic trade
- international trade
- environment.

For each of these six criteria, the consequences were estimated over four geographic levels, defined as:

**Local**: an aggregate of households or enterprises (a rural community, a town or a local government area).

**District**: a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').

**Regional**: a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).

**National**: Australia wide (Australian mainland states and territories and Tasmania).

For each criterion, the magnitude of the potential consequence at each of these levels was described using four categories, defined as:

**Indiscernible**: pest impact unlikely to be noticeable.

**Minor significance**: expected to lead to a minor increase in mortality/morbidity of hosts or a minor decrease in production but not expected to threaten the economic viability of production. Expected to decrease the value of non-commercial criteria but not threaten the criterion's intrinsic value. Effects would generally be reversible.

**Significant**: expected to threaten the economic viability of production through a moderate increase in mortality/morbidity of hosts, or a moderate decrease in production. Expected to significantly diminish or threaten the intrinsic value of non-commercial criteria. Effects may not be reversible.

**Major significance**: expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production. Expected to severely or irreversibly damage the intrinsic 'value' of non-commercial criteria.

Values were translated into a qualitative impact score (A–G)[2] using Table 2.3.

**Table 2.3: Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales**

| | | Geographic scale | | | |
|---|---|---|---|---|---|
| | | Local | District | Region | Nation |
| Magnitude | Indiscernible | A | A | A | A |
| | Minor significance | B | C | D | E |
| | Significant | C | D | E | F |
| | Major significance | D | E | F | G |

The overall consequence for each pest is achieved by combining the qualitative impact scores (A–G) for each direct and indirect consequence using a series of decision rules (Table 2.4). These rules are mutually exclusive, and are assessed in numerical order until one applies.

**Table 2.4: Decision rules for determining the overall consequence rating for each pest**

| Rule | The impact scores for consequences of direct and indirect criteria | Overall consequence rating |
|---|---|---|
| 1 | Any criterion has an impact of '**G**'; or<br>more than one criterion has an impact of '**F**'; or<br>a single criterion has an impact of '**F**' and each remaining criterion an '**E**'. | Extreme |
| 2 | A single criterion has an impact of '**F**'; or<br>all criteria have an impact of '**E**'. | High |
| 3 | One or more criteria have an impact of '**E**'; or<br>all criteria have an impact of '**D**'. | Moderate |
| 4 | One or more criteria have an impact of '**D**'; or<br>all criteria have an impact of '**C**'. | Low |
| 5 | One or more criteria have an impact of '**C**'; or<br>all criteria have an impact of '**B**'. | Very Low |
| 6 | One or more but not all criteria have an impact of '**B**', and<br>all remaining criteria have an impact of '**A**'. | Negligible |

[2] In earlier qualitative IRAs, the scale for the impact scores went from A to F and did not explicitly allow for the rating 'indiscernible' at all four levels. This combination might be applicable for some criteria. In this report, the impact scale of A–F has changed to become B–G and a new lowest category A ('indiscernible' at all four levels) was added. The rules for combining impacts in Table 2.4 were adjusted accordingly.

### 2.2.4 Estimation of the unrestricted risk

Once the above assessments are completed, the unrestricted risk can be determined for each pest or groups of pests. This is determined by using a risk estimation matrix (Table 2.5) to combine the estimates of the probability of entry, establishment and spread and the overall consequences of pest establishment and spread. Therefore, risk is the product of likelihood and consequence.

When interpreting the risk estimation matrix, note the descriptors for each axis are similar (e.g. low, moderate, high) but the vertical axis refers to likelihood and the horizontal axis refers to consequences. Accordingly, a 'low' likelihood combined with 'high' consequences, is not the same as a 'high' likelihood combined with 'low' consequences – the matrix is not symmetrical. For example, the former combination would give an unrestricted risk rating of 'moderate', whereas, the latter would be rated as a 'low' unrestricted risk.

**Table 2.5: Risk estimation matrix**

| Likelihood of pest entry, establishment and spread | Negligible | Very low | Low | Moderate | High | Extreme |
|---|---|---|---|---|---|---|
| **High** | Negligible risk | Very low risk | Low risk | Moderate risk | High risk | Extreme risk |
| **Moderate** | Negligible risk | Very low risk | Low risk | Moderate risk | High risk | Extreme risk |
| **Low** | Negligible risk | Negligible risk | Very low risk | Low risk | Moderate risk | High risk |
| **Very low** | Negligible risk | Negligible risk | Negligible risk | Very low risk | Low risk | Moderate risk |
| **Extremely low** | Negligible risk | Negligible risk | Negligible risk | Negligible risk | Very low risk | Low risk |
| **Negligible** | Negligible risk | Negligible risk | Negligible risk | Negligible risk | Negligible risk | Very low risk |
| | **Consequences of pest entry, establishment and spread** | | | | | |

### 2.2.5 Australia's appropriate level of protection (ALOP)

The SPS Agreement defines the concept of an 'appropriate level of sanitary or phytosanitary protection (ALOP)' as the level of protection deemed appropriate by the WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Like many other countries, Australia expresses its ALOP in qualitative terms. Australia's ALOP, which reflects community expectations through government policy, is currently expressed as represents Australia's ALOP.

## 2.3 Stage 3: Pest risk management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve Australia's ALOP, while ensuring that any negative effects on trade are minimised.

The conclusions from pest risk assessment are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate exceeds Australia's ALOP, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve Australia's ALOP. The effectiveness of any proposed phytosanitary measure (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk, to ensure it reduces the restricted risk for the relevant pest or pests to meet Australia's ALOP.

ISPM 11 (FAO 2004) provides details on the identification and selection of appropriate risk management options and notes that the choice of measures should be based on their effectiveness in reducing the probability of entry of the pest.

Examples given of measures commonly applied to traded commodities include:

- options for consignments – e.g., inspection or testing for freedom from pests, prohibition of parts of the host, a pre-entry or post-entry quarantine system, specified conditions on preparation of the consignment, specified treatment of the consignment, restrictions on end-use, distribution and periods of entry of the commodity
- options preventing or reducing infestation in the crop – e.g., treatment of the crop, restriction on the composition of a consignment so it is composed of plants belonging to resistant or less susceptible species, harvesting of plants at a certain age or specified time of the year, production in a certification scheme
- options ensuring that the area, place or site of production or crop is free from the pest – e.g., pest-free area, pest-free place of production or pest-free production site
- options for other types of pathways – e.g., consider natural spread, measures for human travellers and their baggage, cleaning or disinfestation of contaminated machinery
- options within the importing country – e.g., surveillance and eradication programs
- prohibition of commodities – if no satisfactory measure can be found.

Risk management measures are identified for each quarantine pest where the risk exceeds Australia's ALOP. These are presented in the 'Pest Risk Management' section of this report.

# 3 Pathways

The import of plant commodities provides pathways for the introduction of exotic insect pests and pathogens into new locations. In recent years, '*Candidatus* Liberibacter species' causing Huanglongbing (HLB) disease in citrus and their vectors, *Diaphorina citri* and *Trioza erytreae*, have extended their range around the world. The detection of '*Ca.* L. asiaticus' in some states of USA and the spread of its vector *D. citri* into states that are approved sources of citrus fruit for Australia have raised concerns about the potential introduction of this pathogen and its vector. Import conditions have been in place to allow the importation of citrus fruit from Arizona, California and Texas and fresh leaves from California, USA. In addition, budwood and seed of citrus species has been allowed to be imported through post-entry quarantine and import conditions have been in-place to import fresh leaves of several Rutaceous hosts of '*Ca.* L. species' and their psyllid vectors.

In this PRA, several pathways were identified for the importation of '*Ca.* L. species' and their psyllid vectors. While the probability of entry of the bacterium in the psyllids is considered, the risk profile of the psyllid vectors in association with the various import pathways may differ. Consequently, import pathways for the bacteria and their psyllid vectors are assessed separately. These pathways are described in detail below.

## 3.1 '*Candidatus* Liberibacter species' pathways

In this PRA, information on '*Ca.* L. species' pathogenic to Rutaceous species and their vectors, *D. citri* and *Trioza erytreae*, were reviewed and the following pathways were identified for the introduction of the bacteria into Australia:

- fruit, including seed
- nursery stock (live plants)
- citrus budwood
- fresh leaves
- seed for sowing
- infected psyllids

### 3.1.1 Pathway 1—Fruit containing seed

Citrus fruit is currently permitted entry into Australia from approved sources. Fresh citrus fruit for human consumption is a potential pathway by which '*Ca.* L. species' could be introduced to Australia.

Should infected fruit be imported, the bacterium could possibly be spread to other hosts through intentional or incidental propagation of infected seed.

The risk of entry of '*Ca.* L. species' through potentially infected citrus fruit containing seed is considered in the first of the '*Ca.* L. species' pathway analyses.

Seedless fruit poses equal or less risk than fruit containing seed. Therefore, only fruit containing seed has been considered in the pathway analysis for '*Ca.* L. species' as the conclusions reached for this pathway are equally applicable to the seedless fruit pathway.

### 3.1.2 Pathway 2—Nursery stock (live plants)

Currently, live plants of citrus and other allied genera in the Rutaceae family are not allowed entry into Australia. Nursery stock is a likely avenue for the movement of exotic insects or pathogens to and within Australia. Apart from nursery stock of host species being a pathway for the importation of '*Ca.* L. species', nursery stock could also support all life stages of the pathogens vectors, *D. citri* and *T. erytreae* (see 3.2.2). Infected nursery stock represents one of the most important sources for introducing the bacterium to new areas as nursery stock is imported for the specific purpose of propagation.

While all medium and high risk nursery stock undergoes post-entry quarantine on arrival in Australia, when undertaking the unrestricted risk analysis in this PRA, it is assumed that no quarantine measures have been applied to the nursery stock.

The illegal introduction of nursery stock has a very high risk of introducing '*Ca*. L. species' as the usual quality and quarantine checks are bypassed. However, the assessment of risk posed by potential illegal introductions is outside the scope of this PRA.

The risk of entry of '*Ca.* L. species' pathogenic to Rutaceous species through potentially infected nursery stock is considered in the second of the '*Ca.* L. species' pathway analyses.

### 3.1.3 Pathway 3—Budwood

Citrus germplasm is generally imported as budwood. Budwood consists of a shoot free from foliage, bearing buds suitable for grafting. Budwood is currently permitted entry into Australia, and thus represents a potential pathway by which '*Ca*. L. species' could be introduced to Australia.

Infected budwood represents one of the most important sources for introducing exotic pathogens, including '*Ca*. L. species', as budwood is imported for the specific purpose of propagation (Broadbent 1995).

While all imported budwood undergoes post-entry quarantine on arrival in Australia, when undertaking the unrestricted risk analysis in this PRA, it is assumed that no quarantine measures have been applied to the budwood.

The illegal introduction of budwood has a very high risk of introducing the '*Ca*. L. species' as the usual quality and quarantine checks are bypassed. However, the assessment of risk posed by potential illegal introductions is outside the scope of this PRA.

The risk of entry of '*Ca.* L. species' through potentially infected budwood is considered in the third of the '*Ca.* L. species' pathway analyses.

### 3.1.4 Pathway 4—Fresh leaves

Fresh leaves of several Rutaceous hosts of '*Ca.* L. species' are currently permitted entry into Australia, and thus represents a potential pathway by which the bacterium could be introduced to Australia. Fresh leaves may be imported for all uses other than as animal foods, fertilisers or for growing purposes.

Apart from leaves of host species being a pathway for the importation of '*Ca.* L. species', leaves could also harbour vectors of the pathogens, *D. citri* and *T. erytreae* (see 3.2.4). Should infected leaves be imported, the bacterium could possibly be spread to other hosts through insects feeding on the leaves.

The risk of entry of '*Ca.* L. species' through potentially infected leaves is considered in the fourth of the '*Ca.* L. species' pathway analyses.

### 3.1.5 Pathway 5—Seed for sowing

Seed of citrus species are currently permitted entry into Australia, and thus represents a potential pathway by which the bacterium could be introduced to Australia.

'*Candidatus* Liberibacter species' can be detected at a high level in the seed coats (Tatineni *et al*. 2008). Seeds from HLB affected trees often show various degrees of abortion (Bové 2006), probably related to the presence of the pathogens in the seed coats. Un-aborted seed in infected fruits are characteristically small and brownish/black in colour (Bové 2006).

The risk of entry of '*Ca.* L. species' through potentially infected seed is considered in the fifth of the '*Ca.* L. species' pathway analyses.

### 3.1.6 Pathway 6—Infected psyllids

The citrus psyllids (*Diaphorina citri* and *Trioza erytreae*) are well known, efficient vectors of '*Ca.* L. species' (Bové 2006). Psyllids may be associated with any aerial part of their host plant. Psyllids and their eggs may be present on stems, foliage and fruits. Psyllids have been intercepted on planting material (Sullivan and Zink 2007), fresh leaves (CDFA 2008b; Beattie and Barkley 2009) and fruit (Halbert and Núñez 2004). Therefore, there is potential to introduce infected psyllids into Australia in association with fruit, nursery stock (live plants), budwood or fresh leaves.

Passive transport of adult psyllids, which are strongly attracted to light, in commercial and military aircraft is another avenue for the introduction of citrus psyllids into Australia. However, the assessment of hitchhiker pests in non-trade related pathways is outside the scope of this PRA.

Citrus psyllids have also been introduced into new areas by air movements (e.g. cyclonic and jet streams). Psyllids are capable of long distance spread through active flight, wind-assisted dispersal and through storms and hurricanes (Bové 2006; Tsai 2006; Gottwald *et al.* 2007; Barkley and Beattie 2008). *Diaphorina citri* has been in South America for many years and has spread to Central America and the Caribbean. From there, it is believed to have been carried northward by seasonal trade winds or hurricanes to Florida (Tsai 2006). *Trioza erytreae* is suspected to have spread from Madeira into the Canary Islands via the dominant North-South trade winds (Bové 2006).

Leucaena psyllid (*Heteropsylla cubana*) was introduced into Australia in 1986 from the Western pacific to the northeast of Queensland on air currents associated with severe tropical cyclone Winifred (Bray and Sands 1987). However, the assessment of risk posed by natural introductions is outside the scope of this PRA.

The risk of entry of '*Ca.* L. species' through potentially infected psyllids is considered in the sixth of the '*Ca.* L. species' pathway analyses. This analysis is broken down into four vector pathways outlined below.

## 3.2 Psyllid vector pathways

In this PRA, information on psyllid vectors, *Diaphorina citri* and *Trioza erytreae*, was reviewed and the following pathways were identified for the introduction of these psyllids into Australia:

- fruit
- nursery stock (live plants)
- budwood
- fresh leaves

### 3.2.1 Pathway 1—Fruit

Citrus fruit is currently permitted entry into Australia from approved sources. Fresh citrus fruit for human consumption is a potential pathway by which *T. erytreae* and *D. citri* could be introduced to Australia.

Should infested fruit be imported, the psyllids could possibly be spread to other living hosts through independent dispersal as they are known to actively seek suitable hosts by flight.

The risk of entry of *T. erytreae* and *D. citri* through potentially contaminated citrus fruit is considered in the first of the psyllid vector pathway analyses.

### 3.2.2 Pathway 2—Nursery stock (live plants)

Currently, live plants of citrus and other allied genera in the Rutaceae family are not allowed entry into Australia. However, nursery stock is a likely avenue for the movement of exotic insects or pathogens to and within Australia. Nursery stock is able to support all life stages of the citrus psyllids, *D. citri* and *T. erytreae*. Infested nursery stock represents one of the most important sources for introducing the psyllids to new areas as nursery stock is imported for the specific purpose of propagation.

While all medium and high risk nursery stock undergoes post-entry quarantine on arrival in Australia, when undertaking the unrestricted risk analysis in this PRA, it is assumed that no quarantine measures have been applied to the nursery stock.

The risk of entry of *D. citri* and *T. erytreae* on Rutaceous hosts through potentially infested nursery stock is considered in the second of the psyllid vector pathway analyses.

### 3.1.3 Pathway 3—Budwood

Citrus germplasm is generally imported as budwood. Budwood consists of a shoot free from foliage, bearing buds suitable for grafting. Budwood is currently permitted entry into Australia, and thus represents a potential pathway by which citrus psyllids, *D. citri* and *T. erytreae* could be introduced to Australia.

While all imported budwood undergoes post-entry quarantine on arrival in Australia, when undertaking the unrestricted risk analysis in this PRA, it is assumed that no quarantine measures have been applied to the budwood.

Should infested budwood be imported, the psyllids could possibly be spread to other living hosts through independent dispersal as they are known to actively seek suitable hosts by flight.

The risk of entry of *D. citri* and *T. erytreae* through potentially infested budwood is considered in the third of the psyllid vector pathway analyses.

### 3.2.3 Pathway 4—Fresh leaves

Fresh leaves of several Rutaceous hosts of *D. citri* and *T. erytreae* are currently permitted entry into Australia, and thus represent a potential pathway by which the psyllid vectors could be introduced to Australia. Fresh leaves may be imported for all uses other than as animal foods, fertilisers or for propagation.

Leaves infested by *D. citri* and *T. erytreae* could be imported into Australia. Should infested leaves be imported, the psyllid vectors could possibly be spread to living hosts through independent dispersal as they are known to actively seek suitable hosts by flight.

The risk of entry of *D. citri* and *T. erytreae* through potentially infested leaves is considered in the fourth of the psyllid vector pathway analyses.

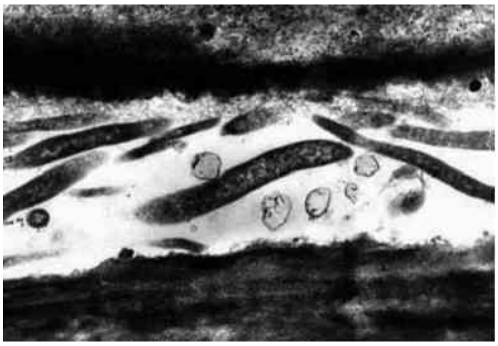
# 4 Pest Information

## 4.1 '*Candidatus* Liberibacter species' associated with the Rutaceae

'*Candidatus* Liberibacter species' associated with the Rutaceae cause Huanglongbing (HLB) disease in citrus and are considered the most damaging vectored transmissible disease of citrus in the world (Bové 2006). Huanglongbing is also known as 'greening' in South Africa, 'mottle leaf' in the Philippines, 'dieback' in India, and 'vein phloem degeneration' in Indonesia (Bové 2006).

The first description of HLB disease symptoms was recorded in India as 'dieback' in the eighteenth century (da Graça 2008). By 1912, the disease was recognised as a serious disease of citrus in several Indian states (da Graça 2008). It was previously thought that HLB symptoms were caused by *Citrus* tristeza virus but it was later shown that the dieback was caused by a bacterium (Gottwald *et al.* 2007). The HLB bacteria are phloem-limited, gram-negative, fastidious α-proteobacteria. The bacteria have thin cell walls that allow them to pass through the narrow sieve pores and survive within the phloem vascular system of a plant (da Graça 2008)(Figure 4.1). '*Candidatus* Liberibacter species' were the first phloem sieve tube restricted bacteria seen in plants (Bové 2006) with the capacity to multiply not only in all types of citrus, but also in the hemolymph and salivary glands of two psyllid vectors: one native to Africa (*Trioza erytreae*) and one native to Asia (*Diaphorina citri*) (Aubert 2008).

**Figure 4.1: '*Candidatus* Liberibacter asiaticus' in sieve tube of sweet orange leaf**



Source (Bové 2006)

Following a naming convention for bacteria that can not be cultured, the HLB bacterium has provisional ('*Candidatus*') status in nomenclature (Jagoueix *et al*. 1996). The bacteria has since been cultured and inoculated to juvenile hosts, resulting in disease symptoms consistent with HLB and partially fulfilling Koch's postulates (Sechler *et al*. 2009).

The following three different '*Ca.* L. species' have been described from citrus:

- '*Candidatus* Liberibacter africanus' Jagoueix *et al*. (1994)
- '*Candidatus* Liberibacter americanus' Teixeira *et al*. (2005a)
- '*Candidatus* Liberibacter asiaticus' Jagoueix *et al*. (1994)

'*Candidatus* Liberibacter species' pathogenic to the Rutaceae can be distinguished on the basis of temperature sensitivity, DNA hybridization and genome properties and serology (Teixeira *et al.* 2005b; Bové 2006; Lopes *et al*. 2009a). Characteristics of these species are summarized in Table 4.1.

**Table 4.1: Characteristics of '*Candidatus* Liberibacter species' pathogenic to Rutaceae**

| | '*Candidatus* Liberibacter species' | | |
|---|---|---|---|
| **Pathogen** | '*Ca*. L. africanus' | '*Ca*. L. asiaticus' | '*Ca*. L. americanus' |
| **Common name** | African HLB | Asian HLB | American HLB |
| **Geographic distribution** | Africa, Mauritius, Réunion, Saudi Arabia, Yemen | Asia, Brazil (states of Minas Gerais, Paraná and São Paulo), Cuba, Dominican Republic, Ethiopia, Mexico, Mauritius, Réunion, Saudi Arabia, USA (Florida, Louisiana, Georgia, South Carolina), Yemen | Brazil |
| **Vector** | *Trioza erytreae* | *Diaphorina citri* | *Diaphorina citri* |
| **Temperature sensitivity** | Heat sensitive | Heat tolerant | Heat sensitive |
| **DNA (base pair)** | 667 | 703 | 1027 |

It has been proposed that '*Ca.* L. species' known to infect the Rutaceae originated in Africa in association with *T. erytreae* and one or more plant species in the genus *Vepris* (Beattie *et al*. 2008). It is considered that the bacterium was present in East Africa in indigenous Rutaceous plants, since citrus is an introduced species in Africa. The psyllid vector (*T. erytreae*) may have transmitted the bacterium from native plants to introduced citrus plants (da Graça 2008; Beattie and Barkley 2009). Then the bacterium was introduced to the Indian sub-continent in infected plants or budwood (Beattie and Barkley 2009) where it adapted to new hosts and environments (Beattie *et al*. 2006).

### 4.1.1 Symptoms of '*Candidatus* Liberibacter species'

'*Candidatus* Liberibacter species' are limited to the phloem, the living tissue that carries carbohydrates to all parts of the plant. Since the bacterium infects phloem, once a plant has become infected the bacterium can move throughout the plant. That is, the infection is systemic, and thus cannot be removed by simply pruning away the part of the tree expressing symptoms (Spann *et al*. 2008). Symptoms caused by '*Ca*. L. species' vary with season (McClean and Schwarz 1970), pathogen species and environmental conditions (Bové 2006) and are not constant over time, within a tree or between locations. Multiple '*Ca.* L. species' can coexist in a single plant. For example, '*Ca.* L. africanus' and '*Ca*. L. asiaticus' in the islands of Réunion and Mauritius (Bové and Ayres 2007; Aubert 2008) and '*Ca*. L. americanus' and '*Ca*. L. asiaticus' in Brazil (Lopes *et al*. 2009b) have been reported from single trees.

Symptoms are most easily detected on leaves, but can also be found on fruits from severely infected trees (Spann *et al*. 2008). Early symptoms in infected *Citrus* species include leaf yellowing on a single shoot or branch (Figure 4.2). Infected leaves show a mottled or blotchy appearance at the initial stage of symptom development. Additionally, leaves may develop vein corking. This symptom is typified by bright yellow leaf veins that are raised and have a corky appearance (Spann *et al*. 2008). With time, the yellowing spreads to other parts of the tree and dieback and rapid decline follow.

Infected trees become stunted, bear multiple off-season flowers (most of which fall off), are sparsely foliated and display yellowing of new shoots (Halbert and Manjunath 2004; Tsai 2006; Polek *et al.* 2007). The affected trees produce fruits that are small, underdeveloped, irregularly shaped, with a thick peel of poor colour turning and remaining green, thus the name 'greening disease' (Halbert and Manjunath 2004; Tsai 2006; Su

2008). Other symptoms include a yellow stain in the peel just below the point of stem attachment, dark-coloured aborted seeds, uneven peel colouring, and a bitter, salty taste.

**Figure 4.2: Foliage symptoms and fruit symptoms of HLB on a) sweet oranges, b) mandarin, c) grapefruit and d) fruit**

(a)

(b)

(c)

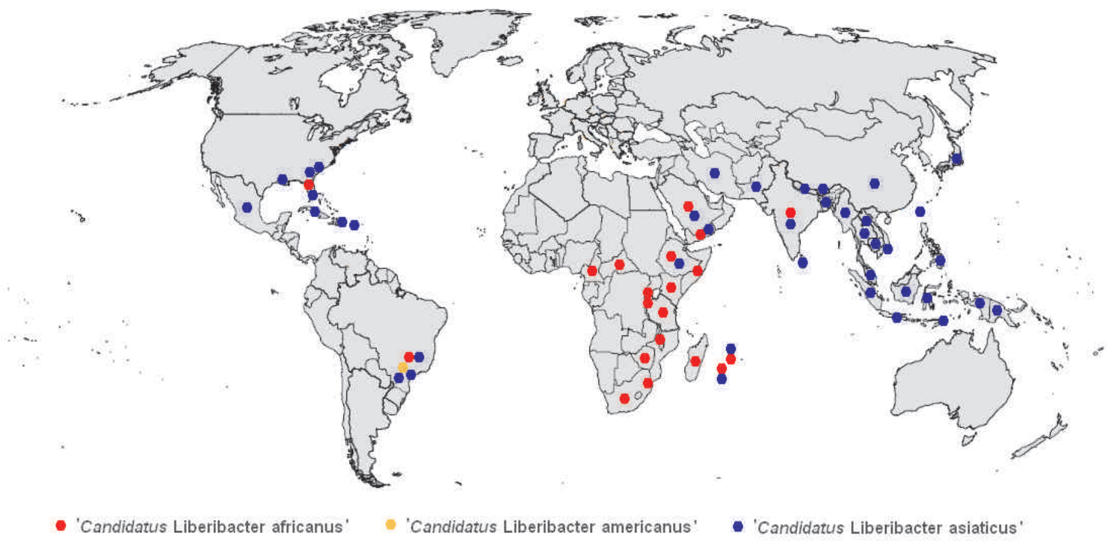
(d)

Source: Bové, IRNA, Bordeaux, France, http://www.doacs.state.fl.us/pi/chrp/greening/cgphotos.html

### 4.1.2 Distribution of '*Candidatus* Liberibacter species'

Citrus infecting '*Ca.* L. species' have long been present in Asia, Africa, the Indian subcontinent, Arabian Peninsula, and the Islands of Mauritius, Réunion and Madagascar (da Graça 1991; Bastianel *et al*. 2005; Faghihi *et al*. 2009) (Figure 4.3).

**Figure 4.3: Distribution of '*Candidatus* Liberibacter species'**




'*Candidatus* Liberibacter asiaticus' has only recently been found in East Timor, Papua New Guinea (Weinert *et al*. 2004), Brazil (Teixeira *et al*. 2005a), Cuba (Llauger *et al*. 2008), Dominican Republic (Matos *et al*. 2009), Mexico (NAPPO 2009a), Puerto Rico (NAPPO 2009b) and the US States of Florida (Li *et al.* 2007), Louisiana (CDFA 2008a), Georgia and South Carolina (USDA 2009), Ethiopia (Saponari *et al*. 2010) Belize (Manjunath *et al.* 2010), and the US Virgin Islands (NAPPO 2010). In mid 2009, the distribution of '*Ca*. L. africanus' was confirmed by molecular techniques in Brazil, India and the state of Florida in the USA (Postnikova *et al*. 2009). To date, '*Ca*. L. species' have not been reported from citrus producing regions of Australia, or countries in Central America or the Mediterranean (Bové 2006).

### 4.1.3 Hosts of '*Candidatus* Liberibacter species'

Known hosts of '*Ca*. L. species' causing HLB and their vectors are presented in Appendix B. There is uncertainty regarding the extent of host susceptibility to '*Ca*. L. species' because of the difficulty in assessing the presence of the pathogen with certainty. Rutaceous plants are the natural hosts of '*Ca*. L. species', with all species and cultivars of citrus susceptible to infection (Beattie *et al.* 2008; Beattie and Barkley 2009). A number of non-Rutaceous species are experimental hosts of '*Ca*. L. species' (Hung *et al*. 2000; Halbert and Manjunath 2004).

### 4.1.4 Transmission of '*Candidatus* Liberibacter species'

'*Candidatus* Liberibacter species' are transmitted by insect vectors, through grafting with diseased budwood (Albrecht and Bowman 2008), through parasitic plant species of *Cuscuta* (Duan *et al*. 2008) and possibly by seed. Even though the pathogens are bacteria, they are not spread by casual contamination of personnel or tools, or by wind and rain.

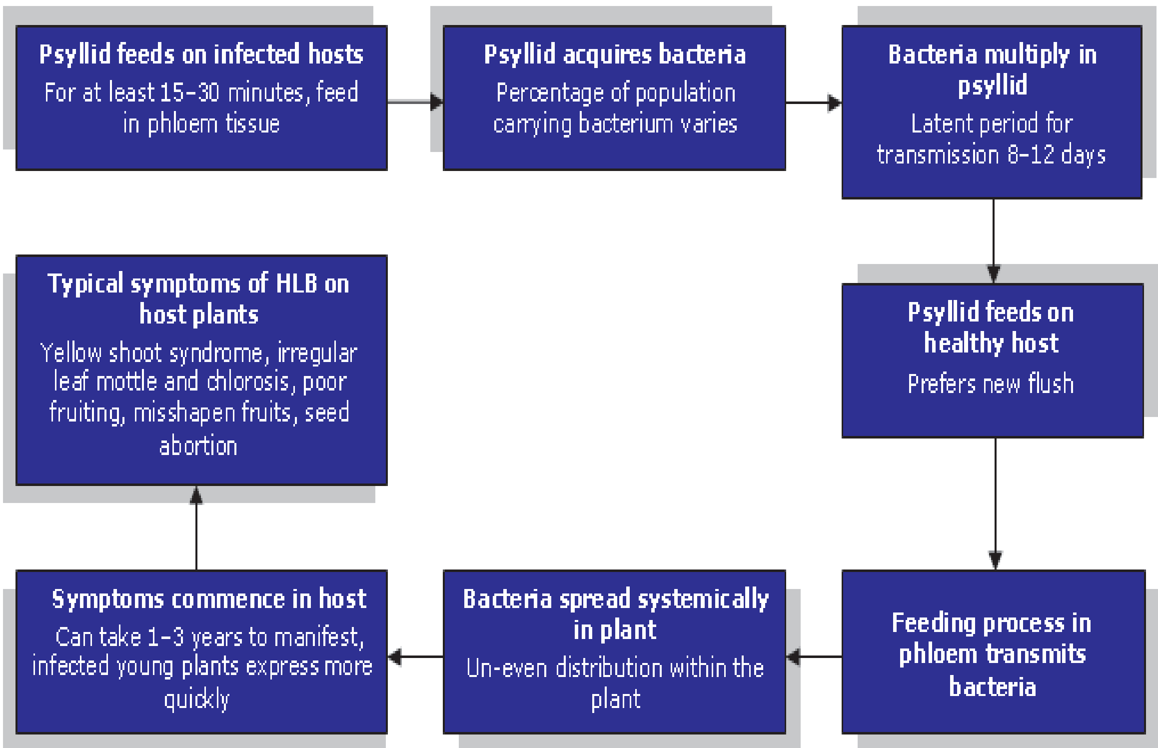
### Psyllid Transmission

These psyllids are citrus pests in their own right in addition to being vectors of '*Ca.* L. species'. Psyllids acquire '*Ca.* L. species' through feeding on infected hosts and are then able to transmit the bacterium to additional hosts as they feed (Bové 2006). Under natural conditions, the following psyllids vector '*Ca.* L. species':

- *Diaphorina citri* vectors '*Ca.* L. americanus' (Teixeira *et al*. 2005b), '*Ca.* L. asiaticus' (Bové 2006) and '*Ca.* L. africanus' (Aubert 2008).
- *Trioza erytreae* vectors '*Ca.* L. africanus' (Bové 2006) and '*Ca.* L. asiaticus' (Aubert 2008).

In Réunion, both psyllids (*D. citri* and *T. erytreae*) and two species of the pathogen ('*Ca*. L. asiaticus' and '*Ca*. L. africanus') occur. *Diaphorina citri* and '*Ca*. L. asiaticus' occur in hot, low-lying areas, while *T. erytreae* and '*Ca*. L. africanus' occur in cool and wet high-lying areas (Aubert 2008). Depending on the season, overlap of psyllid territories is possible. Furthermore, the intermixing of both types of HLB organisms (i.e. African HLB showing symptoms only in cool climates as opposed to Asian HLB that is less temperature dependant) was enhanced by the capacity of each citrus psyllid to transmit either pathogen. Not surprisingly, individual trees were found to host both '*Ca.* L. asiaticus' and '*Ca.* L. africanus' simultaneously (Aubert 2008; Postnikova *et al*. 2009). Transmission of the bacteria by psyllid vectors is presented in Figure 4.4.

**Figure 4.4: Transmission of Huanglongbing by psyllid vectors**




### Graft Transmission

'*Candidatus* Liberibacter species' are graft transmitted (Halbert and Manjunath 2004; Bové 2006; Coletta-Filho *et al.* 2010). However, transmission is variable depending upon the plant parts used for grafting, the amount of tissue used and the bacterial isolate (Bové 2006). Plants grafted with infected buds sourced from symptomatic branches resulted in 100% transmission within 18 weeks (Coletta-Filho *et al.* 2010). Plants grafted with buds from infected, but asymptomatic branches had a transmission rate that ranged from 10 to 60% (Coletta-Filho *et al*. 2010). Studies indicate that the bacteria are unevenly distributed in infected plants (Martinez *et al*. 1971; Huang 1979; Futch *et al*. 2008). After grafting, symptoms may develop after eight weeks in summer and 10 weeks at other times of the year, although some test seedlings took two years or more to develop symptoms (Capoor *et al*. 1974).

'*Candidatus* Liberibacter species' vary in their ability to be graft transmitted. Graft transmission rates in '*Ca.* L. asiaticus' are high (Stover *et al*. 2008; Coletta-Filho *et al*. 2010). Whereas both '*Ca.* L. africanus' and '*Ca.* L. americanus' are poorly transmitted when plants are grafted with buds sourced from symptomatic branches (McClean 1970; Lopes and Frare 2008).

**Dodder (*Cuscuta* spp.) Transmission**

'*Candidatus* Liberibacter species' pathogenic to the Rutaceae have been transmitted to several hosts by dodder (*Cuscuta* species) (Tirtawidjaja 1981; Garnier and Bové 1983; Duan *et al*. 2008). '*Candidatus* Liberibacter asiaticus' has been transmitted from infected citrus to:

- periwinkle (*Catharanthus roseus*) by *Cuscuta australis* (Tirtawidjaja 1981), *Cuscuta campestris* (Garnier and Bové 1983; Ke *et al*. 1988) and an unidentified *Cuscuta* sp. (Subandiyah 1994)
- tobacco (*Nicotiana tabacum*) by *Cuscuta campestris* (Garnier and Bové 1993) and an unidentified *Cuscuta* sp. (Francischini *et al.* 2007)
- tomato (*Solanum lycopersicum*) by *Cuscuta pentagona* (Duan *et al*. 2008)
- orange jasmine (*Murraya paniculata*) by *Cuscuta pentagona* (Zhou *et al*. 2007).

**Seed Transmission**

It has generally been considered that seed transmission of phloem-limited pathogens, such as '*Ca.* L. species' and phytoplasmas, is unlikely because of the lack of direct contact between phloem sieve elements of plants and the developing embryos of seed. There are conflicting reports as to whether '*Ca.* L. species' are seed transmissible in citrus:

- Capoor *et al*. (1974) found that seed transmission did not occur when seedlings were grown from seeds sourced from '*Ca*. L. asiaticus' infected fruit.
- Tirtawidjaja (1981) demonstrated that seeds sourced from HLB affected fruits resulted in few seedlings displaying stunted chlorotic symptoms found in HLB infected hosts.
- Ke *et al*. (1988) found no symptoms in seedlings grown from seeds from HLB trees observed over five years.
- Tatineni *et al*. (2008) found '*Ca*. L. asiaticus' in citrus seed coats but not in the seed endosperm and questioned how the HLB bacterium enters the phloem of seedlings.
- Graham *et al*. (2008) found that seven of 59 seeds sourced from HLB infected citrus fruit tested positive for the bacterium and yielded ~ 700 bp 16s rDNA sequences for '*Ca.* L. asiaticus'. Of the seven plants that originally tested positive for '*Ca*. L. asiaticus', upon re-assay, only three tested positive (Graham *et al*. 2008). Later, when tested a third time, only one of the three formerly positive plants tested positive for the bacteria (Graham *et al*. 2008).
- Shatters (2008) found that seeds from *Citrus sinensis* and *C. paradisi* resulted in few seedlings displaying HLB like symptoms. '*Candidatus* Liberibacter asiaticus' was detected in less than 10% of these seedlings. As plants grew, HLB symptomatic plants developed more slowly than asymptomatic plants, however, most lost HLB symptoms

over time. In this study, Shatters (2008) does not state if the seedlings that lost HLB symptoms remained infected with the bacteria.

- Benyon *et al*. (2009) confirmed the transmission of '*Ca*. L. asiaticus' via seed to seedlings in three *Citrus* species using a range of new sensitive molecular techniques. The authors note that the bacterial titre in the '*Ca*. L. asiaticus' positive seedlings remained very low and most, if not all, of the seedlings did not develop HLB symptoms over a period of three years.

Based on this evidence Plant Biosecurity considers seed derived from infected trees to be a potential pathway for the importation of '*Ca.* L. species'.

**Mechanical Transmission**

Under orchard conditions, mechanical transmission may occur when pathogens are carried unintentionally from an infected tree to a healthy one by way of budding knives, pruning scissors or hedging or topping machines (Bové 1995). However, mechanical transmission of '*Ca.* L. species' has not been reported.

## 4.2 Psyllid vectors

Citrus psyllids, *Diaphorina citri* and *Trioza erytreae*, are the only known natural vectors for the different species of citrus infecting '*Candidatus* Liberibacter' (Halbert and Manjunath 2004). *Diaphorina citri* and *T. erytreae* are able to exploit their environment in a relatively short period of time because of high fecundity of the females, multiple generations per year, high dispersal ability and ability to get to high densities on host plants, thus forming population reservoirs that are difficult to control. Both insects are efficient vectors in HLB contaminated areas (Aubert 1987b). Key biological and morphological differences between *D. citri* and *T. erytreae* are summarised in Table 4.2.

**Table 4.2: Key biological and ecological differences between psyllid vectors**

| ***Diaphorina citri*** | ***Trioza erytreae*** |
|---|---|
|  |  |
| • Efficient vector of '*Candidatus* Liberibacter asiaticus' and '*Ca.* L. americanus'. | • Efficient vector of '*Candidatus* Liberibacter africanus'. |
| • Resistant to high temperatures and survives in hot lower altitudes. | • Sensitive to higher temperature and survives in cool higher altitudes. |
| • Eggs are laid on tips of growing shoots, on and between unfurling leaves.<br>• Eggs are laid along axis vertical to surface | • Eggs are laid along edges of young leaves.<br>• Eggs are laid along axis horizontal to surface. |
| • Nymphs found on young stems and petioles.<br>• Nymphs with massive wing pads.<br>• No gall formed, nymphs completely exposed. | • Nymphs found on lamina of underside of leaves.<br>• Nymphs with small wing pads.<br>• Open gall or pit formed, only dorsal surface of nymph exposed. |
| • Adult wings are with dark areas (maculate). | • Adult wings are clear and transparent. |

### 4.2.1 *Diaphorina citri*

| | |
|---|---|
| **Scientific name** | *Diaphorina citri* Kuwayama [Hemiptera: Psyllidae] |
| **Synonym(s)** | *Euphalerus citri* Kuwayama |
| **Common name** | Asian citrus psyllid, citrus psylla (Asian) |
| **Known hosts** | Rutaceae family |
| **Distribution** | Asia, Africa, Americas |

**Distribution of *Diaphorina citri***

*Diaphorina citri* occurs in the following regions and countries: in Asia, the Arabian Peninsula (Saudi Arabia and Yemen), and from Iran through the Indian Subcontinent, the Indian Ocean Islands of Mauritius and Réunion, Southeast Asia and East Asia, the Philippines and through the Indonesian archipelago to north eastern Papua New Guinea; in Hawaii and Guam; in the USA States of Alabama, California, Florida, Georgia, Louisiana, Mississippi, South Carolina and Texas; as well as Puerto Rico, the Bahamas, Cayman Islands, Cuba, Jamaica, Dominican Republic, Guadeloupe, Venezuela, Brazil, Paraguay, Uruguay and Argentina (Aubert 1987b; Cermeli *et al.* 2000; Halbert and Manjunath 2004; Halbert and Núñez 2004; Weinert *et al*. 2004; EPPO 2009; Conant *et al*. 2009; Poe and Shea 2007; Bové 2006; Mead 2008; CDFA 2008a; Faghihi *et al*. 2009). Recently the United States Department of Agriculture (USDA) confirmed the presence of *D. citri* in Arizona (NAPPO 2009c) and media have reported psyllids are only one mile from commercial citrus groves (Yuma Sun 2009; Examiner 2009).

**Hosts of *Diaphorina citri***

The host range of *D. citri* includes many *Citrus* species and close citrus relatives. All known hosts of *D. citri* are listed in Appendix B. The suitability of hosts is influenced by cultivar, ambient temperature, nutrition, soil moisture, the nutritional value and frequency of flush growth, and local biotypes of the psyllid. Young citrus trees that flush prolifically under ideal conditions are more suitable hosts for the psyllid than older or younger trees grown under poor conditions. *Diaphorina citri*, in contrast to *Trioza erytreae*, is not able to build up massive populations on a wide range of alternative wild rutaceous hosts in natural areas (Aubert 1988). Based on Aubert (1987b) there are at least 21 species of plants on that *D. citri* can feed, but egg laying and nymphal development are restricted to 15 and 14 host species, respectively (Table 4.3). The recent establishment of *D. citri* in Florida, and subsequent survey work to delimit geographical and host range, has expanded this host list. However, the majority of these new hosts do not support complete development of *D. citri* (see Appendix B).

**Table 4.3: Classification of host plants of *Diaphorina citri* (Aubert 1987b)**

| Host classification | Host plant species | Feed and life stage development | | |
|---|---|---|---|---|
| | | Leaf sucking | Egg laying | Nymphal development |
| **Preferred host plants** | *Murraya paniculata* | +++ | +++ | +++ |
| | *Citrus aurantifolia* | +++ | +++ | +++ |
| **Common host plants** | *Citrus limon* | ++ | ++ | ++ |
| | *Citrus sinensis* | ++ | ++ | ++ |
| | *Citrus medica* | ++ | ++ | ++ |
| | *Citrus nobilis* | ++ | ++ | ++ |
| | *Citrus reticulata* | ++ | ++ | ++ |

| Host classification | Host plant species | Feed and life stage development | | |
|---|---|---|---|---|
| | | Leaf sucking | Egg laying | Nymphal development |
| | *Citrus deliciosa* | ++ | ++ | ++ |
| | *Microcitrus australisisaca* | ++ | ++ | ++ |
| | *Citrus paradisi* | ++ | ++ | ++ |
| **Occasional host plants** | *Citrus hystrix* | + | + | + |
| | *Citrus grandis* | + | + | + |
| | *Triphasia trifoliata* | + | + | + |
| | *Fortunella* sp. | + | + | + |
| | *Poncirus trifoliata* | + | + | - |
| | *Murraya koenigii* | + | - | - |
| | *Toddalia asiatica* | + | - | - |
| | *Vepris lanceolata* | + | - | - |
| | *Coriea* sp. | + | Unknown | Unknown |
| | *Atalantia* sp. | + | | |
| | *Clausena lansium* | + | | |

+++ Very common, ++ Usual, + Occasional,
– Not observed in natural nor experimental conditions

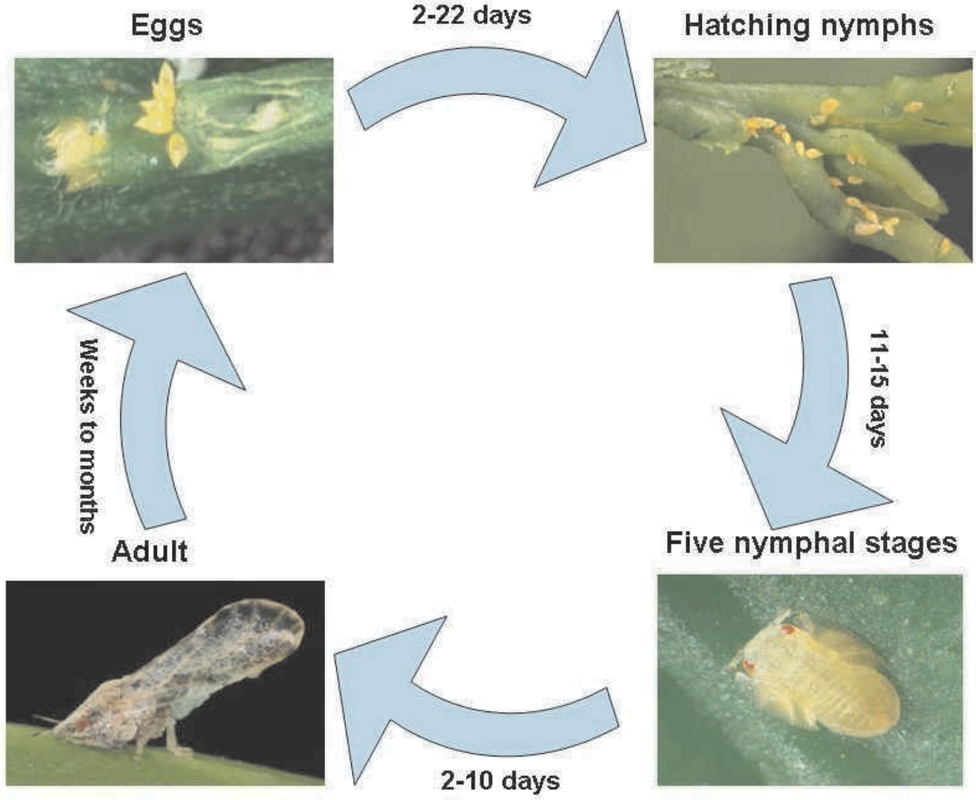
While there are many observations about preferred hosts (Halbert and Manjunath 2004), only a few comparative laboratory studies have been conducted to specifically address the suitability of different host plants on psyllid fitness under controlled conditions. In one study, oviposition was highest on *Murraya paniculata*, and nymphal development was highest on *Citrus limonia* and *M. paniculata* (Nava *et al*. 2007). In this study, nymphal viability was lowest on *Citrus sunki*. In another study, psyllids readily colonized *Citrus aurantium* whereas little or no development was observed on *C. reticulata* (Tsagkarakis and Rogers 2008).

### Biology of *Diaphorina citri*

*Diaphorina citri* thrives in warm environments, and is tolerant of high temperatures (Bové 2006). It is more prevalent in hot coastal areas. The species has a short life cycle and high fecundity (Catling 1973). Eggs are laid singly inside half-folded leaves of the buds, in leaf axils or other suitable places on the young tender parts of the tree (Catling 1970; Pande 1971). Females have a pre-oviposition period of about 12 days and are capable of laying up to 800 eggs during their lives (Mead 1977). Eggs hatch within 2–22 days, depending on temperature, and pass through five instars in 11–25 days according to season (Figure 4.5). The total development time from egg to adult requires 15–47 days, depending on environmental factors such as temperature, season and host quality (Mead 1977; Tsagkarakis and Rogers 2008). The adults may live for several weeks to months (Figure 4.5). There is no diapause, but populations are low in the winter or during dry periods when temperature and host quality limit development. There are 9–10 generations a year, with up to 16 being observed under observation in field cages (CFDA 2008).

The reproductive biology of *D. citri* is closely tied to the availability of new leaf flush for egg laying and subsequent development of nymphs. As *D. citri* is attracted by the volatiles emitted by new shoot growth (Patt and Sétamou 2010), increases in psyllid numbers are thus most evident during periods of abundant new flush (Tsagkarakis and Rogers 2008). *Diaphorina citri* has a strong preference for young shoots, although adult psyllids are also found on older leaves and branches (Bonani *et al*. 2008).

**Figure 4.5: Life cycle of *Diaphorina citri***




All life stages of *D. citri* are vulnerable to extreme temperatures. Freezing temperatures in the range of -2.5 to -5 °C for up to five hours resulted in approximately 40% mortality of eggs. One-hundred percent egg mortality can be achieved if the temperature drops below –5 °C for more than eight or nine hours (Hall 2008b). A temperature of -2.6 °C for up to 10 hours resulted in less than 30% mortality of fifth instar nymphs. Adult psyllids exposed to −5 to −5.5 °C for four hours or longer killed 95–100% of adults. Adult *D. citri* have shown some ability to acclimatise to cold weather (Tsagkarakis and Rogers 2008).

### Damage caused by *Diaphorina citri*

*Diaphorina citri* damages plants directly through its feeding activities on *Citrus* species and closely related ornamentals (Halbert and Manjunath 2004). New shoot growth that is heavily infested by the psyllid does not expand and develop normally, and is more susceptible to breaking off (Grafton-Cardwell *et al.* 2006). Psyllids extract large quantities of sap from the plant as they feed and produce copious amounts of honeydew. The honeydew coats the leaves of the tree, encouraging sooty mould to grow that can reduce photosynthetic activity (Grafton-Cardwell *et al.* 2006).

### 4.2.2 *Trioza erytreae*

| | |
|---|---|
| **Scientific name** | *Trioza erytreae* (Del Guárico) [Hemiptera: Psyllidae] |
| **Synonym(s)** | *Spanioza eritreae* Del Guercio, *Aleurodes erytreae* Del Guercio, *Spanioza erythreae* Del Guercio, *Spanioza erytreae* Del Guercio, *Trioza citri* Laing, *Trioza erythreae* Del Guercio, *Trioza merwei* Pettey |
| **Common name** | African citrus psyllid, citrus psylla (African) |
| **Known hosts** | Rutaceae family |
| **Distribution** | Sub-Saharan Africa |

#### Distribution of *Trioza erytreae*

*Trioza erytreae* has been reported from Angola, Cameroon, Comoros, Republic of the Congo, Democratic Republic of the Congo, Eritrea, Ethiopia, Kenya, Malawi, Rwanda, Sao Tome and Principe, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe (Bové 2006; CABI/EPPO 2006). It also occurs in Saudi Arabia; Yemen; the Mascarene Islands of Madagascar, Mauritius and Réunion; and the Atlantic Ocean islands of Saint Helena, Madeira, Porto Santo, Tenerife and Gomera (Aubert 1987b; Bové 2006; CABI/EPPO 2006).

#### Hosts of *Trioza erytreae*

All known hosts of *T. erytreae* are listed in Appendix B. *Trioza erytreae* is the only species of *Trioza* that is known to feed and develop on the Rutaceae (Hollis 1984; Aubert 1987b). Based on Aubert (1987b) there are at least 18 species of plants on which *T. erytreae* can feed, but egg laying and nymphal development are restricted to 15 and 13 species, respectively (Table 4.4).

**Table 4.4: Classification of host plants of *Trioza erytreae* (Aubert 1987b)**

| Host classification | Host plant species | Feed and life stage development | | |
|---|---|---|---|---|
| | | Leaf sucking | Egg laying | Nymphal development |
| Preferred host plants | *Clausena anisata* | +++ | +++ | +++ |
| | *Vepris lanceolata* | +++ | +++ | +++ |
| | *Citrus limon* | +++ | +++ | +++ |
| | *Citrus medica* | +++ | +++ | +++ |
| | *Citrus aurantifolia* | ++ | ++ | ++ |
| Common host plants | *Citrus sinensis* | ++ | ++ | ++ |
| | *Citrus nobilis* | ++ | ++ | ++ |
| | *Citrus reticulata* | ++ | ++ | ++ |
| | *Citrus deliciosa* | ++ | ++ | ++ |
| | *Citrus paradise* | ++ | ++ | ++ |
| | *Citrus grandis* | + | + | + |
| | *Murraya paniculata* | + | + | + |
| | *Fagara capense* | + | + | + |
| Occasional host plants | *Toddalia asiatica* | + | + | - |
| | *Fortunella species* | + | + | - |
| | *Poncirus trifoliata* | + | - | - |
| | *Calodendron capense* | + | - | - |
| | *Microcitrus australisiaca* | + | - | - |

+++ Very common, ++ Usual, + Occasional

– Not observed in natural nor experimental conditions

*Trioza erytreae* belongs to a complex of species that are difficult to define morphologically, but have discrete host plant preferences (Hollis 1984) (Table 4.5).

**Table 4.5: *Trioza* complex and host plant preferences**

| Species | Host plant family | Host plant species | Distribution |
|---|---|---|---|
| *Trioza ata* Hollis | Salicaceae | *Salix safsaf* | Angola, Tanzania |
| *Trioza capeneri* Hollis | Araliaceae | *Seemannaralia gerrardii* | South Africa |
| *Trioza carvalhoi* Hollis | Araliaceae | *Cussonia angolensis*, *C. paniculata*, *C. spicata* | Kenya, Angola, South Africa, Swaziland |
| *Trioza catlingi* Hollis | Menispermaceae | *Cissampelos* sp., *Stephanania abyssinica* | Kenya, Tanzania, South Africa |
| *Trioza eafra* Hollis | Araliaceae | *Cussonia spicta* | Kenya, Tanzania |
| *Trioza erytreae* (Del Guercio) | Rutaceae | *Clausena anisata*, *Citrus* spp., *Fagara capensis*, *Vepris undulata* | Angola, Cameroun, Ethiopia, Kenya, Madagascar, Malawi, Zaire, Réunion, Ruanda, São Tomé, St Helena, South Africa, Tanzania, Uganda, Zimbabwe, |
| *Trioza gregoryi* Hollis | Unknown | | Nigeria, Burundi, Tanzania |
| *Trioza kilimanjarica* Hollis | Unknown | | Tanzania |
| *Trioza menispermicola* Hollis | Menispermaceae | *Cissampelos owariensis*, *Triclisia macrophylla*, *T. patens* | Ghana, Nigeria |
| *Trioza tiliacora* Hollis | Menispermaceae | *Tiliacora* sp. | Tanzania |

### Biology of *Trioza erytreae*

*Trioza erytreae* is heat sensitive and has similar temperature tolerances to '*Candidatus* Liberibacter africanus' (Schwarz and Green 1970; Catling, 1973). It is very sensitive to extremes of hot, dry weather with the eggs and first instar nymphs being particularly vulnerable (Catling 1969a). Psyllid development is favoured by cool, moist areas over 500–600 m, where citrus growth flushes tend to be prolonged (da Graça 1991). Sex ratios fluctuate in the field, but females always predominate. There is a pre-oviposition period of 3–7 days, but this is considerably extended in the absence of young foliage; longevity is also prolonged under such conditions. Mating occurs 2–4 times a day and eggs may be laid immediately (Van den Berg 1990). Females remain fertile for 11–16 days in the absence of males, and maximum egg production occurs towards the middle of their life span, which normally lasts 17–50 days depending upon season (shorter in summer, longer in winter); up to 1305 eggs may be laid per female (Catling 1973). There is an incubation period of 6–15 days and nymphal development (five instars) takes 1–43 days, both periods being inversely related to mean temperature and directly related to nutritional value of the leaves (Catling 1973). The temperature threshold for nymphal development is approximately 10–12 °C and the species does not undergo diapause.

Over winter, adult psyllids survive on mature leaves of semi-dormant plants (Van den Berg 1990). Although temperatures below 10–12 °C limit nymphal development, adults will continue to reproduce if new flush is available (Van den Berg 1990).

### Damage caused by *Trioza erytreae*

*Trioza erytreae* can cause severe leaf distortion, curling, stunting, galling and chlorosis (EPPO 2005a). *Trioza erytreae* nymphs produce cup-shaped open galls on the under surfaces of leaves of *Citrus* species and other Rutaceae on which they feed (Figure 4.6). Small flush points may be so densely packed with eggs that they may shrivel and fall off (Catling 1972). Chlorosis disappears after the nymphs reach maturity (Van den Berg 1990). The leaves may also be dusted with faecal pellets. These pellets of excrement (honeydew) have the appearance of minute white eggs, and the ground or vegetation under a badly-infested tree may appear as if dusted with white powder (Van der Merwe 1941). The severity of these symptoms is related to levels of infestations.

**Figure 4.6: Nymphs of *Trioza erytreae* make a nest on lower leaf side and produce bumps on upper leaf side**

## 4.2.3 Related psyllids

In addition to *D. citri* and *T. erytreae*, several other psyllid species are known to feed on the Rutaceae, including *Citrus* species where '*Ca.* L. species' are known to occur (Table 4.6). They are not known to be vectors of HLB, but only limited studies have been undertaken to investigate this possibility.

**Table 4.6: Psyllids species recorded on Rutaceae where '*Ca.* L. species' are present**

| Species | Host plant species | Distribution |
|---|---|---|
| *Diaphorina auberti* Hollis | *Citrus* spp. (Halbert and Manjunath 2004) | Comoros (Hollis 1987) |
| *Diaphorina communis* Mather | *Citrus* spp., *Murraya koenigii* (Halbert and Manjunath 2004), *Murraya paniculata* (Mathur 1975). | India (Halbert and Manjunath 2004) |
| *Diaphorina murrayi* Kandasamy | *Citrus* spp., *Murraya exotica* (Halbert and Manjunath 2004) | India (Halbert and Manjunath 2004) |
| *Diaphorina punctulata* Pettey | *Citrus* spp. (Halbert and Manjunath 2004) | Swaziland (Halbert and Manjunath 2004) |
| *Diaphorina zebrana* Capener | *Citrus* (Halbert and Manjunath 2004) | Swaziland (Halbert and Manjunath 2004) |
| *Mesohomotoma lutheri* (Enderlein) | *Citrus* (Aubert and Quilici 1984) | Réunion (Aubert and Quilici 1984) |
| *Psylla murrayi* Mathur | *Murraya koenigii, Citrus* spp. (Mathur 1975; Lahiri and Biswas 1980; Osman and Lim 1990) | India, Malaysia (Halbert and Manjunath 2004) |

| Species | Host plant species | Distribution |
|---|---|---|
| *Psylla citricola* Young & Li | *Citrus grandis, Citrus medica* (Yang and Li 1984) | China (Yang and Li 1984) |
| *Psylla citrisuga* Yang & Li | *Citrus grandis, Citrus medica* (Yang and Li 1984) | China (Yang and Li 1984) |
| *Psylla evodiae* Miyatake | *Murraya paniculata* (Inoue *et al*. 2006) | Japan (Inoue *et al*. 2006) |
| *Trioza citroimpura* Yang & Li | *Citrus reticulata* (Halbert and Manjunath 2004) | China (Halbert and Manjunath 2004) |
| *Trioza litseae* Bordage | *Citrus* spp. (Halbert and Manjunath 2004) | Mauritius, Réunion (Aubert 1987b) |

Considerable variation within widely distributed species of *Diaphorina* can be observed between distant populations of each species. *Diaphorina communis* and other Rutaceae-feeding congeneric species from India may be in fact be synonymous (Burckhardt 1984; 1994; Halbert and Manjunath 2004). *Psylla citricola* and *P. citrisuga* may be closely related to, or synonyms of, *P. murrayi* (Burckhardt 1994). Adults of *P. murrayi* have been observed on citrus, but its presence is considered occasional or incidental, as no eggs or nymphs have been observed on these species (Osman and Lim 1990).

*Diaphorina auberti* can develop on citrus (Hollis 1987); and *D. punctulata* and *D. zebrana* feed on citrus, but are not vectors of '*Ca*. L. africanus' (Catling and Atkinson 1974). The psyllid *Mesohomotoma lutheri* feeds on citrus leaves for short periods, but is extremely rare. Its preferred host is a species of *Hibiscus*. It does not lay eggs on citrus (Aubert and Quilici 1984). *Psylla evodiae* has been reported feeding on *Murraya paniculata* in Japan and it also occurs on *Tetradium glabrifolium* and feeds on *Zanthoxylum beechyanum* var. *alatum* (Inoue *et al*. 2006).

Only one native species of *Diaphorina*, *D. tryoni* (Froggatt) has been recorded from Queensland, Australia (Hollis 2004). Its larvae are free-living and its host is *Conyza viscidula* DC [Asterales: Asteraceae]. Surveys over the past 10 years by CSIRO have failed to detect any psyllids on Aurantioideae in Australia. Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida, T. oleariae* and *T. tristaniae*) are present in Australia, but do not occur on members of the Rutaceae (DEWHA 2009).

The psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta* are found on Rutaceous hosts in Australia (DEWHA 2009). However, it is unlikely that these psyllids would be capable of transmitting '*Ca.* L. species'.

### 4.2.4 Control of psyllids

*Diaphorina citri* can be controlled by insecticidal sprays or by biological control agents. Chemical control is used to control the psyllid in citrus orchards in Réunion and mainland China (Aubert and Quilici 1984; Xu *et al*. 1991). Neem oil and petroleum spray oil have been trialled for use in India and China (Chakravarthi *et al*. 1998; Rae *et al*. 1997). The parasite, *Tamarixia radiata* has been used to control *D. citri* in Florida (Mead 2008; Barkley and Beattie 2008). Other members of Syrphidae and Coccinellidae have been reported to feed on *D. citri*.

Insecticides such as dimethoate and the removal of indigenous hosts in surrounding vegetation can be used to control *T. erytreae* (Van den Berg *et al*. 1991a). In Réunion, *T. erytreae* has been successfully controlled by the introduction of a parasite, *Tamarixia dryi*, from South Africa (Aubert *et al*. 1980).

# 5 Pest risk assessments for quarantine pests

This qualitative pathway risk assessment was initiated due to the expansion in range of '*Candidatus* Liberibacter species' and their vectors, *Diaphorina citri* and *Trioza erytreae*, and the identification of new pathways for their potential entry into Australia.

The results of the pest categorisation are set out in Appendix A. '*Candidatus* Liberibacter species' ('*Ca.* L. africanus', '*Ca.* L. americanus' and '*Ca.* L. asiaticus') and their vectors *Diaphorina citri* and *Trioza erytreae* were identified as quarantine pests for Australia and carried forward for pest risk assessment.

The risk assessments focus on the pathways identified for the potential introduction of '*Ca.* L. species' and their vectors. The probability of entry has been considered individually for each pathway, as the pathway by which '*Ca*. L. species' and their vectors might enter Australia has a significant effect on these assessments. However, the probabilities of establishment and spread and the assessment of potential consequences have been assessed only once for the pathways considered here. This is because the probabilities of establishment and spread and the potential consequences consider post-border issues that are influenced by the susceptibility of hosts, the availability of hosts and the suitability of the environment in Australia for '*Ca.* L. species' and their vectors rather than the pathway of entry.

## 5.1 '*Candidatus* Liberibacter africanus'

'*Candidatus* Liberibacter africanus' known to infect the Rutaceae, originated in Africa in association with *Trioza erytreae* and one or more plant species in the genus *Vepris* (Beattie *et al*. 2008). It is considered that the bacterium was present in East Africa in indigenous Rutaceous plants, and the psyllid vector (*Trioza erytreae*) may have transmitted the bacterium from native plants to introduced citrus plants (da Graça 2008; Beattie and Barkley 2009).

Within '*Ca*. L. africanus' some strain differences are discernible. Isolates from the central Transvaal in South Africa have a higher graft-transmission rate than those from the eastern Transvaal. An isolate from one area induced blotchy-mottle leaf symptoms whereas two others caused symptoms that resembled zinc-like deficiencies (da Graça 1991). A subspecies of '*Ca.* L. africanus', '*Candidatus* Liberibacter africanus subsp. capensis', has been described from the Western Cape region of South Africa from *Calodendrum capensis*, a native Rutaceae in the region (da Graça 2008). The distribution of '*Ca*. L. africanus' is limited by its heat-sensitive nature. However, more recently, mixed populations of '*Ca*. L. africanus' and '*Ca.* L. asiaticus' have been reported from India and Florida, and mixed populations of '*Ca*. L. africanus', '*Ca.* L. americanus' and '*Ca*. L. asiaticus' from Brazil (Postnikova *et al*. 2009). Multiple '*Ca.* L. species' can also coexist within a single plant (Postnikova *et al*. 2009). In a recent survey in South Africa only '*Ca.* L. africanus' was detected in citrus samples from the Western Cape region where both '*Ca*. L. africanus' and '*Ca*. L. africanus subsp. capensis' occur (Pietersen *et al*. 2010).

### 5.1.1 Probability of entry

### Pathway 1—Fruit (containing seed)

Seedless fruit poses equal or less risk than fruit containing seed. Therefore, only fruit containing seed has been considered in the pathway analysis for '*Candidatus* Liberibacter species' as the analysis is also relevant to seedless fruit.

**Probability of importation**

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia with the trade in fresh fruit (containing seeds) for consumption from countries where the pathogen is present is **VERY LOW**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter africanus' is associated with cultivated and native Rutaceae and infects both the plants as well as the fruits (Aubert 1987a), and is therefore associated with the pathway.
- Trees that become infected with '*Ca*. L. africanus' in the nursery or before reaching fruit bearing age tend to have new growth that becomes systemically infected and the trees can fail to produce fruit (Pretorius and Van Vuuren 2006).
- Fruit produced by symptomatic plants may contain the bacterium. Infected fruit are small, lopsided, and have a bitter taste, probably because of higher acidity and lower sugars (da Graça 1991). Many fall prematurely, while those that remain on the tree do not colour properly, remaining green on the shaded side (da Graça 1991), have aborted seed and discoloured vascular bundles (Van den Berg 1990; da Graça 2008). Un-aborted seed are characteristically small and brownish/black in colour (Bové 2006).
- Consumer demand for high quality citrus fruit, including the appearance and shape of the fruit, have been important in raising citrus fruit standards internationally (UNCTAD 2006). The grading of citrus fruit to meet quality standards has been defined by the international standard setting body, Codex Alimentarius. To meet internationally agreed quality standards (Codex 2005), symptomatic fruits from commercial orchards are likely to be removed during grading operations.
- Based on polymerase chain reaction (PCR) testing of fruit parts, the peduncle, columella and seed coat from infected trees were found to contain the bacterium (Tatineni *et al*. 2008). However, the bacterium was not detected in the endosperm and embryo of seed from infected plants (Tatineni *et al*. 2008).
- The bacterium can also be retained in floral parts (petals, pistils and stamens) and could advance to young fruit during fruit development (Tatineni *et al*. 2008). For example, asymptomatic fruits sourced from symptomatic branches were considered to be in the early stages of colonization, as '*Ca.* L. species' were only detected in the peduncle and central axis (Li *et al*. 2009). The bacterium was not detected in the pericarp, mesocarp, endocarp or locular membranes of the pre-symptomatic fruits (Li *et al*. 2009).
- The peduncle of fruit harvested from symptomatic trees is known to carry high concentrations of the bacterium (Tatineni *et al*. 2008). However, high levels of peduncle infection are likely to be associated only with symptomatic fruit.
- Generally the bacterium is detected only in symptomatic fruits. Fruit is known to harbour lower concentrations of the bacterium (Floyd and Krass 2006). For example, '*Ca.* L. species' were detected in symptomatic fruits, but the population levels were 1000-fold less than the populations in the midribs, leaf blades, bark and roots (Li *et al*. 2009).
- The detection of '*Ca*. L. species' in fruit harvested from asymptomatic branches has not been recorded.

- The distribution of '*Ca.* L. species' within the host tissues is variable (Tatineni *et al*. 2008), with the level of infection in symptomatic fruit being comparatively low (Li *et al*. 2009). Based on this knowledge, the level and incidence of bacterial infection of asymptomatic fruit, particularly from healthy trees, is likely to be low.

***Ability of the pest to survive transport and storage***

- '*Candidatus* Liberibacter africanus' survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006) and congeneric species '*Ca.* L. asiaticus' can survive cold storage to 4 °C for two months (Li *et al.* 2008).
- Fruit destined for Australia would be shipped in refrigerated containers maintained at 4–6 ºC. These storage conditions are unlikely to have any impact on the survival of the bacterium in imported fruit.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter africanus' is typically managed by the removal and destruction of infected trees, since the bacterium is systemic and cannot be eliminated (Pretorius and Van Vuuren 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The abortion of most infected fruits, obvious symptoms of infection, international quality standards that exclude fruit damaged by pests and no known history of interception in fruit supports an assessment of 'very low'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed in a viable state within Australia with imported fruit (containing seed) from countries where the pathogen is present and transferred to a suitable host, is **EXTREMELY LOW**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter species' are unable to move independently to a suitable host. Movement of '*Ca*. L. africanus' to a suitable host is only possible either by grafting or by psyllid vectors (Van Vuuren 1993). Fruit and fruit parts are not suitable for grafting.
- Without grafting, the movement of '*Ca.* L. africanus' from imported fruit to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. africanus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- Although five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, none of these species have been recorded on the Rutaceae (DEWHA 2009).
- Furthermore, even if known psyllid vectors are present in Australia, they preferentially feed on new flush (Van den Berg 1990) and are unlikely to feed on fruits. Currently, there is no information on the ability of psyllid vectors to transmit '*Ca.* L. africanus' from infected host fruit to healthy host plants.
- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). These

hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.

***Distribution of the imported commodity in the PRA area***

- Imported fruit will be distributed throughout Australia as wholesalers and retailers are located at multiple locations. Distribution of infected fruit would facilitate the distribution of '*Ca*. L. africanus'.
- '*Candidatus* Liberibacter africanus' would need to survive transportation and storage within the PRA area. Fruit is typically stored and transported in refrigerated containers maintained at 4–6 ºC. The bacterium survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Thus, transport and storage conditions are unlikely to have any impact on the survival of '*Ca.* L. africanus' bacteria in imported fruit distributed for retail or market sale.

***Risks from by-products and waste***

- Although the intended use of fresh fruit is human consumption, waste material would be generated (e.g. overripe and damaged fruit, uneaten portions). Whole or parts of the fruit may be disposed of at multiple locations throughout Australia in compost bins or amongst general household or retail waste.
- '*Candidatus* Liberibacter species' have been detected in infected fruit parts including the peduncle, columella, seed coat and fruit peel (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete phloem-limited bacteria. For example, composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. africanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008).
- A relatively high proportion of household and retail waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. species' from the household and environment, reducing the likelihood that susceptible plants will be exposed to this bacterium.
- If psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on fruit waste.
- Seeds present in fruit waste will desiccate quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, seed may not germinate because they do not tolerate moisture loss.
- If a plant was to establish from seed disposed of in a compost bin or amongst general household waste, the seedlings would then be subject to ambient conditions, including variable moisture, temperature extremes (heat and frost) and plant competition, which will reduce seedling survival. Furthermore, seed to seedling transmission would be critical for the bacterium to enter the environment.
- Seed to seedling transmission has been reported based on symptoms (Tirtawidjaja 1981). However, as '*Ca.* L. species' are found only in the seed coat and not in the seed endosperm and embryo, it has been questioned how the bacterium enters the phloem of seedlings (Tatineni *et al*. 2008).
- Recent research suggests that while a small proportion of seedlings that develop from the seed of infected plants are positive for the bacterium, the infection rate can decrease over time (Graham *et al*. 2008).

- In the absence of a suitable vector the bacterium would be unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

The desiccation of citrus seed, the variable and likely unsuitable conditions for seedlings, transient seed transmission and inability of '*Ca.* L. africanus' to be distributed in the absence of a vector or graft transmission supports an assessment of 'extremely low'.

## Pathway 2—Nursery stock (live plants)

### Probability of importation

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia with trade in nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter africanus' has been reported in association with cultivated and native Rutaceous species and can be associated with all vegetative parts of host plants (Aubert 1987a; Aubert 2008). Therefore, nursery stock of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter species' can have a long latent period and many infected plants remain symptomless, particularly those that are newly infected (McClean and Oberholzer 1965b; Catling and Atkinson 1974). It is highly likely that nursery stock from countries where this bacterium is present will contain the bacterium.
- '*Candidatus* Liberibacter africanus' survives within the living phloem cell, in the vascular system of host plants (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant, which other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).
- Since '*Ca*. L. species' infect phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). Symptom expression is influenced by temperature and varies with season (McClean and Schwarz 1970). In unfavourable environments symptoms may not be expressed (Bové 2006).
- Symptoms may include leaf yellowing on a single shoot or branch, a mottled or blotchy appearance and yellowing spreading to other parts of the tree (Van den Berg 1990; Bové 2006). Therefore, symptomatic nursery stock would most likely be detected on arrival in Australia.
- '*Candidatus* Liberibacter species' can be detected in asymptomatic host foliage only when the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008). However, '*Ca.* L. species' are difficult to detect because they are often present at a low concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter africanus' is heat-sensitive (Aubert 1987a) and the onset of visual symptoms can be quite variable depending upon temperatures (McClean and Oberholzer 1965b; Catling and Atkinson 1974). Higher temperatures may suppress symptom development and therefore the bacterium may escape detection (Bové 2006).
- Infected nursery stock may be asymptomatic in the initial stages of infection and can be easily overlooked. For example, it is reported that 15–20% of HLB infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- Association with nursery stock can provide long term survival for '*Ca*. L. species'. The bacteria have a long incubation period and many infected plants remain symptomless, particularly those that are newly infected (McClean and Oberholzer 1965b; Catling and

Atkinson 1974). It is likely that asymptomatic plants infected by '*Ca.* L. africanus' would pass routine visual inspections and be released into Australia.

***Ability of the pest to survive transport and storage***

- Nursery stock is expected to be shipped at moderate temperatures and humidity levels to ensure nursery stock survival; conditions that are unlikely to adversely affect any '*Ca*. L. africanus' present during shipment.
- '*Candidatus* Liberibacter africanus' has been introduced into new areas through the importation of infected planting material (Pretorius and Van Vuuren 2006).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter africanus' is typically managed by the removal and destruction of infected trees, since the bacterium is systemic and cannot be eliminated (Pretorius and Van Vuuren 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The association of '*Ca*. L. africanus' with asymptomatic nursery stock, the likelihood that the bacterium would remain viable during transport and storage and the introduction of this bacterium into new areas with nursery stock supports an assessment of 'high'.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed within Australia with imported nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter africanus' arriving in Australia with nursery stock will not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host (Oberholzer *et al*. 1965; Pretorius and Van Vuuren 2006).
- Natural movement of '*Ca*. L. species' from infected nursery stock to a suitable host, in the absence of vectors, is limited or absent (Polek *et al.* 2007). The bacterium can move to a suitable host only by grafting (Aubert 2008).
- Without grafting, the transfer of '*Ca.* L. africanus' from imported nursery stock to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. africanus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Nursery stock is imported specifically for the purpose of propagation. Infected nursery stock is therefore likely to be planted directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported nursery stock.
- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to these hosts in the PRA area.
- Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, but none of these species have been recorded on the Rutaceae (DEWHA 2009).

- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to transmit this bacterium.
- In the absence of a suitable vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- The distribution of nursery stock would be for retail distribution to multiple destinations throughout Australia. Nursery stock would also be distributed to multiple locations for propagation. Distribution of infected nursery stock commercially through nurseries would facilitate the distribution of '*Ca*. L. africanus'.
- Infected nursery stock may be distributed to glasshouses or retail shops where the bacterium may continue multiplying within the host. Infected nursery stock is unlikely to be grown in isolation, providing greater opportunity for the bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected nursery stock to other hosts.
- '*Candidatus* Liberibacter africanus' would need to survive transportation and storage within the PRA area. Nursery stock is expected to be maintained at moderate temperatures and humidity levels to ensure nursery stock survival, so a portion of infected nursery stock that enters the country is likely to reach areas of host abundance.
- As nursery stock may not display obvious symptoms of '*Ca.* L. africanus' infection, there is a risk that infected plant material would be used for propagation. Material from infected plants may be used for grafting and grafted rootstock is planted directly at multiple locations in Australia.
- Asymptomatic plants may also be overlooked and sold to commercial users and households.

***Risks from by-products and waste***

- Although the intended use of nursery stock is for propagation, and all imported material would be grown under ideal conditions, waste material may be generated. Whole or parts of the plants may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures, that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. africanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However, acquisition and transmission times for *T. erytreae* can take 1–21 days from living host tissue (Moll and Martin 1974; Van Vuuren and Van der Merwe 1992).

The association of '*Ca.* L. africanus' with asymptomatic nursery stock and the likely distribution of infected plants for propagation support an assessment of 'high' for the distribution of this species in nursery stock.

## Pathway 3—Budwood

### Probability of importation

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia with trade in budwood from countries where the pathogen is present is **MODERATE**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter africanus' has been reported in association with cultivated and native Rutaceous species and can be associated with all vegetative parts of host plants (Aubert 1987a; Aubert 2008). Therefore budwood could provide a pathway for the importation of '*Ca.* L. africanus' into Australia.
- '*Candidatus* Liberibacter africanus' survives within the living phloem cell, in the vascular system of host plants (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant, which other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).
- Since '*Ca*. L. species' infect phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). However, the bacterium has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present in all stems of infected trees.
- Infected field trees can be identified by their foliar and fruit symptoms (Polek *et al*. 2007). Budwood is high value and a large investment is made in the health and survival of mother plants. Therefore, it is unlikely that budwood would be sourced from symptomatic trees.
- '*Candidatus* Liberibacter species' can have a long latent period and many infected plants remain symptomless, particularly those which are newly infected (McClean and Oberholzer 1965b; Catling and Atkinson 1974). It is therefore likely that budwood sourced from asymptomatic trees could contain low concentrations of the bacterium (Huang *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter species' can be detected in asymptomatic hosts only when the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008). However, '*Ca.* L. species' are difficult to detect because they are often present at a low concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter africanus' is heat-sensitive (Aubert 1987a) and the onset of visual symptoms can be quite variable depending upon temperatures and varies with season (McClean and Oberholzer 1965b; McClean and Schwarz 1970; Catling and Atkinson 1974). In unfavourable environments (e.g. high temperatures) symptoms may not be expressed (Bové 2006).
- Infected mother plants may be asymptomatic in the initial stages of infection and can be easily overlooked when sourcing budwood. For example, it is reported that 15–20% of HLB infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- Generally, visible symptoms are not produced on budwood and may escape detection during routine visual inspection. Association with budwood can provide long term survival for the bacterium.

***Ability of the pest to survive transport and storage***

- Budwood is expected to be shipped at moderate temperatures and humidity levels to ensure survival; conditions which are unlikely to adversely affect any '*Ca*. L. africanus' present during shipment.
- '*Candidatus* Liberibacter africanus' has been introduced into new areas through the importation of infected planting material (Pretorius and Van Vuuren 2006).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter africanus' is typically managed by the removal and destruction of infected trees, since the bacterium is systemic and cannot be eliminated (Pretorius and Van Vuuren 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

Although '*Candidatus* Liberibacter africanus' is associated with budwood and the bacterium would remain viable during transport and storage, the bacterium is unevenly distributed within host trees and may not be present in budwood sourced from infected asymptomatic hosts. Therefore, an assessment of 'moderate' for the importation of this species in budwood is allocated.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed within Australia with imported budwood from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter africanus' arriving in Australia with imported budwood will not need to move from the import pathway to a suitable host as the bacterium is already within a suitable host (Oberholzer *et al*. 1965; Pretorius and Van Vuuren 2006).
- '*Candidatus* Liberibacter africanus' is unable to move independently from imported budwood to a suitable host. Movement of '*Ca*. L. africanus' to a suitable host is only possible either by grafting or by psyllid vectors (Van Vuuren 1993).
- Budwood is imported specifically for the purpose of grafting onto local rootstock, which will then be planted directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the grafted rootstock. Therefore, the bacterium will move from imported budwood to a suitable host by grafting.
- Graft transmission has been demonstrated for '*Ca*. L. africanus' (Van Vuuren 1993). However, graft transmission is variable depending on the bacterial titre, the plant part used for grafting and the amount of tissue used (Van Vuuren 1993). Therefore, the grafting technique and low bacterial titres will affect symptom expression and could prevent early detection.
- The bacterium can be transmitted by grafting; however, transmission rates can be reduced due to necrosis in sieve tubes (McClean and Oberholzer 1965a) and uneven distribution of the bacterium (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).
- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

- '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. africanus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids, *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to transmit this bacterium.
- Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, but none of these species have been recorded on the Rutaceae (DEWHA 2009).
- In the absence of a suitable vector the bacterium is unable to move from grafted plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- Imported budwood would be distributed to multiple destinations throughout Australia for propagation. Distribution of infected budwood commercially would facilitate the distribution of '*Ca*. L. africanus'.
- Plants grafted with imported infected budwood may be distributed to greenhouses or retail shops where the bacterium may continue multiplying within the host. Grafted plants are unlikely to be grown in isolation, providing greater opportunity for this bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to other hosts.
- Asymptomatic plants resulting from grafting with imported budwood may also be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter africanus' would need to survive transportation and storage within the PRA area. Grafted plants are expected to be maintained at moderate temperatures and humidity levels to ensure their survival, so a high portion of infected plants are likely to reach areas of host abundance.

***Risks from by-products and waste***

- Although the intended use of budwood is for propagation, and all imported material is likely to be used, waste material may be generated. Whole or parts of the budwood may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing the bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. africanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However,

acquisition and transmission times for *T. erytreae* can take 1–21 days from living host tissue (Moll and Martin 1974; Van Vuuren and Van der Merwe 1992).

The association of '*Ca*. L. africanus' with budwood and the likely distribution of infected budwood and grafted plants support an assessment of 'high' for the distribution of this species in budwood.

## Pathway 4—Fresh leaves

### Probability of importation

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia with trade in fresh leaves from countries where the pathogen is present is **MODERATE**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter species' can be associated with all vegetative parts of host plants including foliage (Tatineni *et al.* 2008). Therefore, fresh leaves of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter africanus' survives within the phloem vascular system of the host plants (Bové 2006). Although the bacterium infects phloem, the bacterium has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present in all leaves of infected trees.
- Symptom expression is influenced by temperature and varies with season (McClean and Schwarz 1970). In unfavourable environments symptoms may not be expressed (Bové 2006). Therefore asymptomatic foliage may be imported into Australia.
- Symptoms of leaves infected by '*Ca.* L. species' include a mottled or blotchy appearance at the initial stage of development and vein corking, which is typified by bright yellow leaf veins that are raised and have a corky appearance (Spann *et al.* 2008). Therefore, symptomatic leaves would most likely be detected on arrival in Australia.
- Infected plants may be asymptomatic in the initial stages of infection and could be easily overlooked when harvesting leaves for export.
- The bacteria have a long incubation period and many infected plants remain symptomless, particularly those which are newly infected (McClean and Oberholzer 1965b; Catling and Atkinson 1974). It is likely that asymptomatic leaves infected by '*Ca.* L. africanus' would pass routine visual inspections.

#### *Ability of the pest to survive transport and storage*

- Fresh leaves destined for Australia would be air freighted and, during transportation and storage, kept at low temperatures and high humidity to ensure the material remains fresh. These moderate conditions are unlikely to have any impact on the survival of '*Ca.* L. africanus' in imported fresh leaves.

#### *Ability of the pest to survive existing pest management procedures*

- '*Candidatus* Liberibacter africanus' is typically managed by the removal and destruction of infected trees, since the bacterium is systemic and cannot be eliminated (Pretorius and Van Vuuren 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

'*Candidatus* Liberibacter africanus' is associated with fresh leaves, the foliage of infected plants may remain asymptomatic and the bacterium is likely to remain viable during transport and storage. However, the bacterium is unevenly distributed within host trees and may not be present in the leaves sourced from infected hosts. Therefore an assessment of 'moderate' for the importation of this species in fresh leaves is allocated.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed within Australia in a viable state with imported fresh leaves from countries where the pathogen is present and transferred to a suitable host is **EXTREMELY LOW**.

#### *Ability of the pest to move from the pathway to a suitable host*

- '*Candidatus* Liberibacter africanus' is unable to move independently from imported fresh leaves to a suitable host. The bacterium is able to move to a suitable host only by grafting or by psyllid vectors (Van Vuuren 1993). Cut leaves are not suitable for grafting.
- Without grafting, the transfer of '*Ca.* L. africanus' from imported fresh leaves to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. africanus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Although five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, none of these species have been recorded on the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids, *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to transmit this bacterium.
- Furthermore, even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on cut leaves.

#### *Distribution of the imported commodity in the PRA area*

- Asymptomatic leaves will be distributed for all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption (e.g. curry leaves).
- Fresh leaves may be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the produce is likely to reach areas of host abundance.
- '*Candidatus* Liberibacter africanus' would need to survive transportation and storage within the PRA area. Fresh leaves are likely to be kept at low temperatures and high humidity to ensure the material remains fresh. These moderate conditions are unlikely to have any impact on the survival of '*Ca.* L. africanus' in imported fresh leaves distributed for retail or market sale.

#### *Risks from by-products and waste*

- Although the intended use of fresh leaves includes all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption, waste material would be generated. Fresh leaves may be disposed of at multiple locations throughout Australia in compost bins or amongst general waste.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures, that can be lethal to a range of

pathogens (Noble and Roberts 2004), and '*Ca*. L. africanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008).

- A relatively high proportion of household and retail waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. species' from the household and environment, reducing the likelihood that susceptible plants will be exposed to this bacterium.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste.

Although '*Ca.* L. africanus' is associated with fresh leaves, the bacterium is unable to disperse unassisted, is unlikely to be transmitted by a vector even if known vectors are present in Australia (as vectors prefer to feed on fresh foliage) and the pathway is unsuitable for graft transmission. Therefore an assessment of 'extremely low' is given for the distribution of this species in fresh leaves.

## Pathway 5—Seed for sowing

### Probability of importation

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia with trade in seed of Rutaceous species for sowing from countries where the pathogen is present is **LOW**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter species' have been detected in citrus seed coats, but not in the seed endosperm and embryo (Li *et al*. 2009; Tatineni *et al*. 2008). Therefore this bacterium is associated with the pathway.
- Seeds from '*Ca.* L. species' infected trees often show various degrees of abortion (Bové 2006), probably related to the presence of the pathogens in the seed coats.
- Based on research into seed to seedling transmission at low levels (Benyon *et al*. 2008; Graham *et al*. 2008; Hartung *et al*. 2008; Shatters 2008) the USA banned the importation of seed for sowing from species of known host genera in the family Rutaceae from countries where citrus infecting '*Ca.* L. species' are present (APHIS 2008b).
- A proportion of fruit on infected trees is aborted and the remaining fruit has a high rate of aborted seed (Halbert and Manjunath 2004). Un-aborted seed in infected fruits are characteristically small and brownish/black in colour (Bové 2006). Such seeds may be rejected due to quality issues.
- The distribution of '*Ca.* L. species' within the host tissue is variable, with the level of infection in symptomatic fruit being comparatively low (Tatineni *et al*. 2008; Li *et al*. 2009).
- Asymptomatic fruit, harvested from symptomatic branches, have tested positive for the bacteria (Li *et al*. 2009). The detection of '*Ca*. L. species' in fruit harvested from asymptomatic branches has not been recorded. Based on this knowledge, the level and incidence of bacterial infection in seed from asymptomatic fruit, particularly from healthy trees, is likely to be low.

#### *Ability of the pest to survive transport and storage*

- '*Candidatus* Liberibacter africanus' survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006) and congeneric species '*Ca.* L. asiaticus' can survive cold storage to 4 °C for two months (Li *et al.* 2008).

- Citrus seed would be shipped in refrigerated containers maintained at 3–7 ºC (Willits and Newcomb Inc 2004). These storage conditions are unlikely to have any impact on the survival of the bacterium in imported seed for sowing.
- Citrus seed is highly perishable and sowing should occur as soon as possible following importation. Temperature extremes and exposure to direct sunlight may be fatal to citrus seeds (Willits and Newcomb Inc 2004). Thus, the length of time taken to transport seed from the country of origin, and the time it spends in storage prior to export, may lower its viability and that of any associated bacterium.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by the removal and destruction of infected trees, since the bacterium is systemic and cannot be eliminated (Pretorius and Van Vuuren 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The lack of fruit produced by infected trees, the high rate of aborted seeds and the discolouration of the seeds supports an assessment of 'low' for the importation of '*Ca*. L. africanus' in seed.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed within Australia in a viable state with imported seed of species of Rutaceae from countries where the pathogen is present is **MODERATE**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter africanus' arriving in Australia with imported seed may not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host. '*Candidatus* Liberibacter species' can be detected at a high level in the seed coats (Tatineni *et al*. 2008).
- Unlike seed imported in fruit, seed for sowing is imported specifically for the purpose of propagation and can be a significant investment for importers. Infected seed is therefore likely to be sown directly into suitable habitats at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported seed.
- Although, '*Ca.* L. africanus' is unable to move independently from imported seed to a suitable host, the bacterium is able to move from the imported seed to the resultant seedling.
- Seed to seedling transmission has been reported when seed sourced from infected plants were grown (Tirtawidjaja 1981; Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008). However, the infection rate can decrease over time and symptom expression can be lost completely (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- Recent experimental studies have confirmed the seed to seedling transmission of '*Ca*. L. species' (Benyon *et al*. 2009); however, over a three year period the bacterial titre remained at very low levels and most, if not all, of the seedlings did not develop typical HLB symptoms (Benyon *et al*. 2009).
- There is no report of seed transmission in *Murraya* species or any other citrus relative. However, seed transmission (as high as 53%) of a related species '*Ca*. L. asiaticus' was recently reported experimentally for dodder and periwinkle (Zhou *et al*. 2008b).

- If an infected seedling does establish, insect vectors would be required to move the bacterium to new hosts.
- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.
- In the absence of a vector the bacterium is unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacterium may not persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- The distribution of seed for sowing would be for commercial and retail distribution to multiple destinations throughout Australia. Seed for sowing would also be distributed throughout the PRA area for propagation.
- Seeds would be imported to introduce new genetic material to commercial citrus production. Imported citrus seeds are therefore likely to be distributed to suitable areas where citrus is grown.
- Distribution of infected seed commercially through nurseries would facilitate the distribution of '*Ca*. L. africanus'. Asymptomatic plants that develop from infected seed may also be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter africanus' would need to survive transportation and storage within the PRA area. Citrus seed would be transported and stored in refrigerated containers maintained at 3–7 ºC (Willits and Newcomb Inc 2004). '*Candidatus* Liberibacter africanus' survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Thus, transport and storage conditions are unlikely to have any impact on the survival of '*Ca.* L. africanus' bacteria in imported seed.

***Risks from by-products and waste***

- The intended use of seed is for propagation, and all imported seed would be grown under ideal conditions. Citrus seed that does not survive transportation and storage may shrivel and discolour, and may not be used for sowing. Therefore, waste material may be generated. As the bacterium can infect the entire plant (Tatineni *et al*. 2008) any such material that is discarded may contain the bacterium.
- Seeds of most *Citrus* species desiccate quite quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, discarded seed may not germinate because they do not tolerate moisture loss.
- If a seed does germinate and seed to seedling transmission occurs, an infected plant will establish; however, the infection rate can decrease over time and symptom expression is completely lost (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- The transfer of '*Ca.* L. africanus', from an infected seedling established at a waste depot or in the backyard, to a host would require a vector. The known vectors are not present in Australia.
- Since '*Ca*. L. species' are spread only by a vector or grafting, it is highly likely that an infected plant, which develops in an area where the psyllid vector is not known to occur, will represent an isolated infection and the plant will ultimately die (Floyd and Krass 2006).

Although, '*Ca.* L. species' are associated with seeds at variable rates, the low level of seed to seedling transmission and loss of symptoms over time, support an assessment of 'moderate' for the distribution of '*Ca.* L. africanus' in seeds.

## Pathway 6—Infected Psyllid

### Probability of importation

The likelihood that '*Candidatus* Liberibacter africanus' will arrive in Australia in an infected psyllid vector (*Trioza erytreae*)[3] from countries where the pathogen and psyllid is present is **MODERATE**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter africanus' is vectored primarily by *Trioza erytreae* (Bové 2006), although transmission by *Diaphorina citri* has been reported (Aubert 2008).
- '*Candidatus* Liberibacter africanus' is naturally associated with *T. erytreae* (Aubert 1987b; Van den Berg 1990) and the bacterium has been detected in the salivary glands and salivary ducts of the psyllid (Aubert 1987b). Therefore this bacterium is associated with the pathway.
- *Trioza erytreae* feeding on infected host plants will acquire the bacterium. Once the psyllids have acquired bacterium they maintain the bacteria and the ability to spread the bacterium throughout their life (Gottwald *et al.* 2007) as the bacterium can survive persistently in *T. erytreae* (Bové 2006).
- Large numbers of '*Ca.* L. africanus' have been observed inside *T. erytreae* by electron microscopy (Moll and Martin 1974), which may suggest that the bacterium multiplies inside the insect (da Graça 2008).
- Infections of the psyllid by the bacterium, multiplication of the bacterium in the psyllid and transovarial transmission are important components of interaction between '*Ca*. L. africanus' and *T. erytreae* (Bové 2006; Rogers *et al*. 2008).
- It has been reported that *T. erytreae* nymphs are unable to acquire '*Ca*. L. africanus' (McClean and Oberholzer 1965a; Catling 1969b; Moll and Martin 1974; Catling and Atkinson 1974). However, more recently it has been demonstrated that a small proportion of second–fifth instar nymphs can acquire the bacterium (Van den Berg *et al*. 1991–1992). Therefore, '*Ca*. L. africanus' could be associated with second–fifth instar nymphs as well as the adults.
- Transovarial transmission of '*Ca*. L. africanus' by *T. erytreae* has also been reported. Therefore, '*Ca*. L. africanus' is associated with eggs of *T. erytreae* and can infect all life stages of the psyllid (Van den Berg *et al*. 1991–1992).
- The proportion of infective psyllids in a given psyllid population is variable. Studies conducted in South Africa indicated that the number of adult *T. erytreae* carrying '*Ca*. L. africanus' is relatively small compared to the total adult population in a region where incidence of the disease was high (McClean 1974).
- Seasonal fluctuations in the number of infected psyllids has also been reported: more occurring among adults emerging from the summer flushes on citrus trees, and few, if any, among those emerging during winter and early spring. However, the efficacy of the vector was not seriously impaired by the small number of infected psyllids (McClean 1974).

---

[3] Although both *Trioza erytreae* and *Diaphorina citri* are reported to vector '*Ca.* L. africanus' in Réunion (Aubert 2008), only *T. erytreae* is considered here as it is the primary vector.

- *Trioza erytreae* feeds and breeds on a wide range of plants within the Rutaceae (Hollis 1984; Aubert 1987b). It would be possible for infected psyllids to enter Australia via trade in commodities, such as fresh fruit, nursery stock, budwood and fresh leaves, of suitable host species (Refer to Chapter 5.5).
- Leaf material or trash in imported fruit is a significant risk for the entry of all life stages of *T. erytreae* as eggs are laid on the leaves and nymphs and adults feed under surfaces of leaves (Catling 1972).
- A related species, *Trioza litseae*, has been reported on citrus from Mauritius and Réunion (Aubert 1987b) where '*Ca*. L. africanus' has been present since the 1960s (da Graça 1991). However, this psyllid has not been reported to vector '*Ca*. L. africanus' (Halbert and Manjunath 2004).

***Ability of the pest to survive transport and storage***

- Once infected with '*Ca.* L. species', psyllids remain infective for life (Gottwald *et al.* 2007). '*Candidatus* Liberibacter africanus' is therefore able to survive for long periods in infected psyllids provided the conditions are suitable for the survival of the psyllids.
- Infected psyllids are likely to enter Australia via trade in fresh fruit, nursery stock, budwood and fresh leaves, of suitable host species (Refer to Chapter 5.5) and must survive the transport and storage conditions for these commodities in order to vector '*Ca.* L. africanus'.
- *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988). The death of the psyllids under field conditions, where temperatures reached 27 °C and relative humidity dropped to 37%, was attributed to desiccation rather than starvation (Van den Berg and Deacon 1988). Therefore infected psyllids could survive for longer periods during transportation and storage under low temperatures and high humidity.
- Infected psyllids associated with fresh leaves or nursery stock have the potential to continue feeding during transport and storage.
- The threshold temperature for nymphal development is 10–12 °C (Van den Berg 1990) and the commodities they are likely to be in association with will be shipped at low (4–6 °C for fruit) to moderate temperatures. Transport temperatures are not lethal to *T. erytreae* but are expected to slow development. Furthermore, the psyllid is known to remain infective over winter, at altitude, in temperate South Africa (Van den Berg 1990).
- '*Candidatus* Liberibacter africanus' also survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Low temperatures during transport and storage are unlikely to have any impact on the survival of '*Ca.* L. africanus' bacteria within the psyllid.
- There is no reason to suspect that the duration of transport and storage would lower the viability of '*Ca*. L. africanus' in *T. erytreae*.

***Ability of the pest to survive existing pest management procedures***

- *Trioza erytreae* numbers are often suppressed in Southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006), and a range of generalist insect predators (Van den Berg 1990). These control methods will reduce but not necessarily eliminate *T. erytreae* carrying '*Ca*. L. africanus'. These methods will have no impact of the level of '*Ca.* L. africanus' infection in surviving psyllids.

The ability of '*Ca.* L. africanus' to infect *Trioza erytreae* persistently, association of the psyllid with host plants, variable number of infected psyllids and the likelihood of the bacterium remaining viable within the psyllid during transport and storage supports an assessment of 'moderate'.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter africanus' will be distributed within Australia with infected psyllid to suitable hosts from countries where the pathogen is present is **HIGH**.

### *Ability of the pest to move from the pathway to a suitable host*

- '*Candidatus* Liberibacter africanus' arriving in Australia with infected *T. erytreae* may continue to multiple inside the psyllid vector (da Graça 2008).
- *Trioza erytreae* adults are able to transmit '*Ca*. L. africanus' (Van den Berg 1990). A small proportion of fourth and fifth instar nymphs are reportedly able to transmit the pathogen (Van den Berg *et al*. 1991–1992), but they are vulnerable to desiccation (Aubert 1987b), therefore reducing the risk of spread of the bacterium with nymphs.
- For '*Ca.* L. africanus' to be transferred from the psyllid to a plant host, infected psyllids would need to complete their development to the adult stage (if they are immature), find a host to feed on and successfully transmit the bacterium to the host.
- Immature stages of the psyllid must have suitable food sources to allow them to survive until they reach adulthood. However, eggs and young instars are extremely vulnerable to desiccation (Aubert 1987b). Both '*Ca.* L. africanus' and *T. erytreae* do not tolerate hot and dry climates (Aubert 1987b) and would therefore have trouble surviving distribution within the PRA area to the more arid to semi-arid climates with low rainfall and high temperatures (Aubert 1987b).
- Although they are considered weak fliers, adults of *T. erytreae* are capable of moving independently at least 1.5 km (Van den Berg and Deacon 1988) and are able to survive up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant (Van den Berg and Deacon 1988). Therefore *T. erytreae* carrying '*Ca*. L. africanus' is capable of locating potential host plants to transmit the bacterium.
- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). *Trioza erytreae* feed and reproduce on the Rutaceae (Halbert and Manjunath 2004; Van den Berg 1990), including cultivated *Citrus* species and Australian naturalised/native species *Murraya paniculata* and *Citrus australasica* (Aubert 1987b). There is significant overlap between the host range of the bacterium and the psyllid suggesting the psyllid is likely to distribute the bacterium to a suitable host.
- Suitable hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- Once a suitable host has been found, infected adult psyllids are able to transmit the pathogen at high rates, and transmission success increases the longer they feed on a host (Van Vuuren and Van der Merwe 1992).

### *Distribution of the imported commodity in the PRA area*

- Psyllids carrying '*Ca.* L. africanus' imported into Australia on fruit, fresh leaves, nursery stock and budwood may be distributed throughout the PRA with these commodities as the commodities are imported for wholesale and retail sale to multiple locations.

- Infected *T. erytreae* would need to survive transportation and storage within the PRA area. The threshold temperature for nymphal development is 10–12 °C (Van den Berg 1990). Cold storage is unlikely to be lethal to *T. erytreae* but is expected to slow development. It is considered unlikely that transportation and storage temperatures would reduce or eliminate '*Ca.* L. africanus' within the psyllid since the bacterium survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). The psyllid is known to remain infective over winter, at altitude, in temperate South Africa (Van den Berg 1990).
- After extended periods (> 60 days) without suitable foliage for oviposition *T. erytreae* can still oviposit hundreds of viable eggs once suitable foliage is located (Catling 1969a).

***Risks from by-products and waste***

- Infected psyllids are likely to be distributed throughout the PRA area with fresh fruit, fresh leaves, and budwood and nursery stock of Rutaceous hosts. Although the intended use of these commodities is for human consumption (fruit, fresh leaves) and propagation (budwood and nursery stock), waste material would be generated. Whole or parts of the imported fruit, fresh leaves, budwood or nursery stock infested or contaminated with life stages of the psyllid may be disposed of at multiple locations throughout Australia in compost bins or amongst general household waste.
- Infected psyllids may survive on waste material for a short time before dispersing to suitable hosts. *Trioza erytreae* adults are able to survive from 55 to 85 hours without feeding (Catling 1973; Van den Berg and Deacon 1988) and are able to fly significant distances (Aubert 1987b; Van den Berg *et al.* 1991a).
- Immature stages of the psyllid are extremely vulnerable to desiccation (Aubert 1987b) and adults do not tolerate hot and dry climates (Aubert 1987b) and therefore would have trouble surviving distribution to the more arid to semi-arid climates with low rainfall and high temperatures (Aubert 1987b).

The association of '*Ca*. L. africanus' with *Trioza erytreae*, the ability for infected psyllids to disperse both independently and through the movement of infested host commodities, the host range overlap of the two species (psyllid and the bacterium), and the presence of multiple hosts within the PRA area support an assessment of 'high' for the distribution of '*Ca*. L. africanus' in *Trioza erytreae*.

## Overall probability of entry (importation x distribution)

The overall probability of entry of '*Ca*. L. africanus' is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2). The overall probability of entry for the six pathways being assessed in this PRA is set out in Table 5.1.

**Table 5.1: Overall probability of entry of '*Ca*. L. africanus' on different pathways**

| Pathway | Probability of importation | Probability of distribution | Overall probability of entry |
|---|---|---|---|
| Fruit (containing seed) | Very low | Extremely low | Extremely low |
| Nursery stock (Live plants) | High | High | High |
| Budwood | Moderate | High | Moderate |
| Fresh leaves | Moderate | Extremely low | Extremely low |
| Seed for sowing | Low | Moderate | Low |
| Infected psyllids | Moderate | High | Moderate |

### 5.1.2 Probability of establishment

The likelihood that '*Candidatus* Liberibacter africanus' will establish within Australia, based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is **HIGH**.

***Availability of suitable hosts, alternative hosts and vectors in the PRA area***

- Rutaceous plants are the natural hosts of '*Ca*. L. africanus', with all species and cultivars of citrus susceptible to infection (Aubert 1987a; Van den Berg 1990). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- The bacterium has also been recorded on *Calodendrum capensis*, *Toddalia asiatica* and *Vepris lanceolata* (Rutaceae: Aurantioideae: Rutoideae), which are native to South Africa (Aubert 1987a; Van den Berg 1990). These hosts are not present in Australia.
- '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. africanus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008), which could provide a means for the establishment of this bacterium if introduced into Australia.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids, *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to transmit this bacterium.
- Although five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, none of these species have been recorded on the Rutaceae (DEWHA 2009).
- In the absence of a vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacterium may fail to persist in the environment (Floyd and Krass 2006).

***Suitability of the environment***

- '*Candidatus* Liberibacter africanus' has established successfully in the citrus growing regions of the east African coast to the Red Sea and Yemen. '*Candidatus* Liberibacter africanus' has been reported from Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Réunion, Rwanda, Saudi Arabia, Somalia, South Africa, Swaziland, Tanzania, Yemen and Zimbabwe (EPPO/CABI 1990). The climatic regions across this range are diverse and there are similar climatic regions in parts of Australia that would be suitable for the establishment of '*Ca*. L. africanus'.
- '*Candidatus* Liberibacter africanus' is heat sensitive and has been reported only in the cooler areas of its distribution (Aubert 1987b; Bové 2006). For example, in Kenya, Madagascar, Mauritius, Réunion and Yemen the bacterium occurs only in the cool highlands (Bové 2006).
- '*Candidatus* Liberibacter africanus' symptoms are more pronounced in somewhat moist, cool conditions and at high elevations, than in the low-lying hot areas, and are also more pronounced in winter (Schwarz and Green 1972; Polek *et al*. 2007).
- In Africa, '*Ca*. L. africanus' is most severe in cool areas above 600 m and where relative humidity rarely falls below 25%. At lower altitudes the disease is less severe, and is practically absent below 200 m (Bové *et al.* 2008). Only in the Cape Town region, at the most southern tip of Africa does the disease occur at sea level because latitude compensates for altitude (Bové *et al.* 2008).

- Optimum temperature for the establishment and expressing of symptoms of '*Ca.* L. africanus' is 22–24 °C, while higher temperature (above 32 °C) may suppress the symptoms (Bové 2006). '*Candidatus* Liberibacter africanus' is therefore more likely to establish in the temperate southern regions of Australia and not in the subtropical and tropical northern regions.

***The reproductive strategy and survival of the pest***

- '*Candidatus* Liberibacter africanus' multiplies and survives in the phloem of infected host plants (Pretorius and Van Vuuren 2006) and in the haemolymph and salivary glands of its psyllid vector, *Trioza erytreae* (Aubert 2008).
- '*Candidatus* Liberibacter species' are reliant on infected host plants for survival and will survive as long as the infected plant survives. Symptom development may be delayed for several months, or as long as 2–3 years after initial infection (Capoor *et al*. 1974; Hung *et al*. 2001; Gottwald *et al*. 2007). '*Candidatus* Liberibacter species' destroy citrus orchards within 5–8 years where they occur (Gottwald *et al*. 1989). Therefore the bacteria would reproduce and survive for this period of time in infected plants.
- The survival and multiplication of this bacterium within its host is affected by temperature. '*Candidatus* Liberibacter africanus' survives and produces symptoms at 22–24 °C while high temperatures (above 32 °C) may suppress the symptoms (Bové 2006).
  - Plants graft inoculated with '*Ca.* L. africanus' from Africa produced symptoms at 22–24 °C, whereas no symptoms occurred at 27–32 °C.
  - Another study demonstrated that sweet orange plants severely affected by '*Ca*. L. africanus' exposed to warm temperatures (27–32 °C) quickly produced new, vigorous growth, recovered, and remained symptomless (Bové 2006).
  - In South Africa, '*Ca.* L. africanus' caused severe crop losses in Northern Province and Mpumalanga, presumably due to the upsurge of its vector, *T. erytreae*. Damage was particularly severe in cooler areas > 700 m above sea level where humidity rarely falls below 25% and mean monthly maximum temperatures vary between 18 °C and 30 °C (Beattie *et al*. 2008).
  - These studies explain why in Africa, Madagascar, Réunion Island, Mauritius Island and Yemen the disease is seen only in cool elevated areas.
- Outside a host plant, '*Ca.* L. africanus' can multiply and survive in its psyllid vectors for as long as the vector is alive. *Trioza erytreae* adults are known to survive 73–82 days under experimental conditions (Van den Berg 1990). In the field, adults overwinter on semi dormant trees, and may survive for two to three months on mature foliage (Van den Berg 1990). '*Candidatus* Liberibacter africanus' is therefore able to survive for long periods in infective psyllids.

***Cultural practices and control measures***

- There are currently no control measures in commercial orchards that target '*Ca* L. africanus' and no programs exist for garden, amenity or native hosts. Therefore, current control measures are unlikely to impact on the establishment of '*Ca* L. africanus' in Australia.
- In South Africa, '*Ca*. L. africanus' is controlled by using disease free planting material which has been certified as grown in psyllid free nurseries (Pretorius and Van Vuuren 2006).
- Systemic insecticides are applied to control the vector and severely infected trees, or infected branches are removed, to control the disease (Pretorius and Van Vuuren 2006). However, many chemicals known to be effective against *T. erytreae* overseas are not

currently registered in Australia (Beattie and Barkley 2009). Therefore, chemical control measures are unlikely to impact on the establishment of '*Ca* L. africanus' in Australia.

'*Candidatus* Liberibacter africanus' is able to survive and multiply within suitable hosts and its psyllid vectors, and survives in diverse climatic conditions supports an assessment of 'high' for the establishment of '*Ca.* L. africanus' in Australia.

### 5.1.3 Probability of spread

The likelihood that '*Candidatus* Liberibacter africanus' will spread based on a comparison of factors in the area of origin and in Australia that affect the expansion of the geographic distribution of the pest, is: **HIGH (VERY LOW[4])**.

***The suitability of the natural or managed environment for natural spread***

- '*Candidatus* Liberibacter africanus' may have originated in Africa and has spread throughout southern Africa, along the east African coast to the Red Sea and Yemen (da Graça 2008). There are similarities in the natural and urban environments of these areas with those in Australia (Peel *et al.* 2007), which suggests that '*Ca*. L. africanus could be capable of spread within Australia.
- The bacterium is typically limited to temperate regions and is restricted to cooler highlands in countries of hot-arid to tropical climates and is inactivated at temperatures above 32 °C (da Graça 1991; Bové 2006). Therefore, this species would have trouble spreading in the warm arid and tropical regions of Australia.
- Host plants that support the spread of '*Ca*. L. africanus' are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment. Hosts include all species and cultivars of citrus and some non-citrus Rutaceae (Aubert 1987a; Van den Berg 1990; da Graça 2008). The bacterium has also been recorded on *Calodendrum capensis*, *Toddalia asiatica* and *Vepris lanceolata* (native to South Africa), but these hosts are not present in Australia.
- Natural spread of '*Ca*. L. species' is dependent on the transmission of the bacterium by its psyllid vectors (Rogers *et al* 2008). Natural spread, in the absence of vectors, is limited or absent (Polek *et al.* 2007).
- In the absence of vectors, spread of '*Ca.* L. species' would rely on the movement of infected propagative material (Polek *et al.* 2007). However, the bacterium itself is unable to spread from infected plants to new hosts; infected plants will ultimately senesce and the pathogen will fail to persist in the environment (Floyd and Krass 2006).

***Presence of natural barriers***

- '*Candidatus* Liberibacter africanus' is heat-sensitive (Bové 2006) and hot arid regions surrounding many citrus production areas in Australia would prove to be a significant natural barrier to the spread of this bacterium.
- Hosts of '*Ca.* L. africanus' are present in many parts of Australia (APNI 2008). Natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances between suitable hosts in parts of Australia, may prevent long-range natural spread of this bacterium.
- The Australian citrus industry is spread across Australia in isolated regions with long distances often separating commercial orchards. This will aid in containment and

[4] In the absence of suitable psyllid vectors

eradication of the pathogen and/or vector if establishment occurs in an isolated citrus growing region (Barkley and Beattie 2008).

- A significant natural barrier to spread is the absence of suitable vectors of the bacterium in the PRA area. Natural spread of '*Ca*. L. africanus' is achieved by *T. erytreae* and *D. citri*, which are not present in Australia.

***Potential for movement with commodities or conveyances***

- '*Candidatus* Liberibacter africanus' could be spread to new areas through the movement of infected propagative material (Aubert 1987a; Bové 2006). As visual symptoms may not be present, and in the absence of specific testing regimes, infected nursery stock could easily be moved to new areas.
- The introduction of infected plant material establishes the pathogen in new areas and, if unregulated movement occurs, it will accelerate the spread of the pathogen (Gottwald *et al*. 2007).
- Graft transmission has been demonstrated for '*Ca*. L. africanus' (Van Vuuren 1993). However, graft transmission is variable depending on the plant part used for grafting and the amount of tissue used (Van Vuuren 1993; Halbert and Manjunath 2004).
- With single buds, graft transmission of '*Ca*. L. africanus' varied from 0–50%, depending upon the isolate used (Van Vuuren 1993). Side grafts with twigs are even more efficient at transmitting the pathogen, whereas fruit stems and bark strips were not effective (Van Vuuren 1993).
- '*Candidatus* Liberibacter africanus' does not readily pass to progeny trees propagated by buds from infected trees (McClean 1970), possibly because of necrosis of sieve tubes (McClean and Oberholzer 1965a) and uneven distribution of the bacterium (Huang 1979). However, greater transmission occurs when scions are developed from larger propagative parts such as a piece of twig top-grafted to seedlings (McClean 1970).
- A difference in graft transmissibility of isolates of '*Ca.* L. africanus' from different citrus growing regions have also been reported. However, this variation may be due to titres of the organism within the tissue, which could be influenced by climate (Van Vuuren 1993). A higher transmissibility rate indicates a higher titre of the organism, which will favour transmission (Van Vuuren and Van der Merwe 1992).
- There is also potential for the spread of the bacterium through infected seed. However, research has demonstrated that while a proportion of seedlings that develop from seed from infected plants are positive for the bacterium and a proportion of these positive seedlings develop symptoms of citrus greening, the infection rate can decrease over time and symptoms can be lost completely (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- Infected psyllid vectors, could spread the bacterium through contaminating commodities and conveyances. *Trioza erytreae* can spread the bacterium through transport of infested plant material (Gottwald *et al.* 2007; Van den Berg 1990).

***Potential natural enemies***

- '*Candidatus* Liberibacter africanus' is not known to have any natural enemies that could hamper its spread. However, certain entomophagous insects can drastically reduce vector populations and thus indirectly prevent an increase of the pathogen biomass and associated spread (Aubert 1987a).

**Spread potential if known vectors establish in Australia[5]**

- '*Candidatus* Liberibacter africanus' is vectored primarily by *Trioza erytreae* (Bové 2006) although transmission by *Diaphorina citri* has been reported (Aubert 2008).
- '*Candidatus* Liberibacter africanus' and *Trioza erytreae* are both heat sensitive (Bové 2006) and sub-tropical climates of high lying areas are suitable for the spread of the vector and the pathogen (Aubert 1987b; Van den Berg 1990).
- Both the pathogen and the psyllid are unable to spread to arid or semi-arid climates with low rainfall and high temperatures. Therefore the spread of the pathogen and the psyllid is likely to be limited to the temperate, most southern regions of Australia and not to the subtropical and tropical northern regions (Barkley and Beattie 2008).
- *Trioza erytreae* feeding on infected host plants will acquire the bacterium and once the psyllids have acquired bacterium they maintain the bacterium and the ability to spread the bacterium throughout their life (Gottwald *et al.* 2007).
- The natural spread of '*Ca*. L. africanus' by psyllid vectors will depend on acquisition period, latency of the pathogen in the psyllid prior to transmission and transmission efficiency (Rogers *et al* 2008). There are vast differences reported in the literature regarding these components of the vector-pathogen interaction.
  - The acquisition and latent period to transmit the bacterium was initially reported to take 21 days (Moll and Martin 1974). Van Vuuren *et al*. (1986) showed transmission times as short as two days are possible.
  - A more detailed study found *T. erytreae* adults are able to acquire the bacterium within one hour of feeding (Van Vuuren and Van der Merwe 1992). A latent period of 3–4 days is required before transmission occurred after acquisition feeding for one, two and four hours. However, no latent period is required after acquisition feeding for eight and 24 hours (Van Vuuren and Van der Merwe 1992).
  - Adults are able to transmit the pathogen at a high rate within seven days after a one day acquisition feeding on the infected plants (Van Vuuren and Van der Merwe 1992). For example, transmission was low (4–12 %) when adults, irrespective of the time they feed on infected seedlings to acquire the pathogen, were allowed up to four days to transmit the pathogen to healthy plants, but increased to 32% when they were allowed to transmit the pathogen over seven days (Van Vuuren and Van der Merwe 1992).
- A small proportion of fourth and fifth instar nymphs are reportedly able to transmit the pathogen (Van den Berg *et al*. 1991–1992), but they are vulnerable to desiccation (Aubert 1987b), therefore reducing the risk of spread of the bacterium with nymphs.
- Adults of *T. erytreae* have a lifespan of 2–3 months on host plants in cooler environments (Van den Berg 1990). It is therefore likely that the psyllids would survive long enough in the environment to become infective and spread the bacterium. However, the adults are heat sensitive and will not spread the bacterium in areas with temperatures of 32 °C or more combined with 30% relative humidity (Aubert 1987b).

---

[5] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca*. L. africanus' in the presence of known vectors. Known psyllid vectors, which are currently absent from Australia, can enter and establish in Australia independent of the bacterium through trade in host commodities, conveyances and through natural spread (see Chapter 3). Consequently, Plant Biosecurity has assessed spread both in the presence and absence of known vectors.

- The climatic conditions predicted to limit the spread of *T. erytreae* in Australia are the hot, dry conditions towards inland Australia. The psyllid thrives only in cool environments, and is sensitive to high temperatures combined with low humidity (Bové 2006).
- Green and Catling (1971) show the combined effects of temperature and humidity (measured as the saturation deficient index) and have mapped the distribution and prevalence of *T. erytreae* in South Africa through time. Only regions or seasons with high humidity and moderate temperatures resulted in high populations of *T. erytreae*.
- The effect of climate on the spread of *T. erytreae* is best illustrated in Réunion Island in the Indian Ocean. The climate of Réunion is tropical, with temperatures affected by elevation. The average coastal temperature is between 18–31 °C, with temperatures dropping in the interior; humidity is high. Here, *T. erytreae* co-exists with *D. citri* (da Graça 1991). *Trioza erytreae* is restricted to altitudes above 500 m where the climate is cooler while *D. citri* occurs below this altitude where the climate is warmer (da Graça 1991).
- Overall, Australian climatic conditions in the southern commercial horticultural growing areas would support the development and survival of *T. erytreae* and associated bacteria (Barkley and Beattie 2008).
- The presence of natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances between suitable hosts, may limit the ability of *T. erytreae* to spread '*Ca*. L. africanus' to new areas.
- *Trioza erytreae* is capable of moving independently at least 1.5 km (Van den Berg and Deacon 1988).
- Bass Strait may act as a natural barrier to *T. erytreae* carrying '*Ca.* L. africanus' from mainland Australia to Tasmania as the psyllids would not be capable of dispersing the distance over the strait. However, *T. erytreae* has the potential to move large distances (more than tens of kilometres) on seasonal trade winds or hurricanes and is reported to have spread from the Canary Islands to Madeira in this way (Tsai 2006; Bové 2006).
  - Passive movement of the psyllid indicates that infected psyllids could overcome the natural barriers in Australia and spread the bacterium to suitable environments.
- Suitable hosts of *T. erytreae* and the bacterium are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- Once '*Ca.* L. species' are detected in a citrus orchard, in the absence of control measures, known vectors have been recorded to spread the pathogen to 100% of trees in five years (Bové 2006).

The temperature preference of both the bacterium and the known psyllid vector, presence of multiple host species throughout the PRA area, potential to disperse in propagative material and potential for spread with vectors supports an assessment of 'high' for the spread of '*Ca.* L. africanus'.

**Spread potential in the absence of known vector[6]**

- In the absence of known vectors, the most likely means of spread of '*Ca.* L. africanus' would be through the movement of infected planting material. If infected budwood is used for grafting it will help spread the bacterium to non-infested areas.

[6] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca.* L. africanus' in the absence of known vectors.

- If '*Ca*. L. africanus' was to establish in Australia it would likely lead to the death of its infected host plants and not pose a severe threat to the citrus industry (Barkley and Beattie 2008).
- Although there are five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) present in Australia none of them occur on the Rutaceae (DEWHA 2009). Therefore these species are unlikely to act as vectors.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids, *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to act as vectors.

In the absence of *T. erytreae* from Australia, the assessment supports a rating of 'very low' for the spread of '*Ca.* L. africanus'.

### 5.1.4 Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probability of entry, of establishment and of spread using the matrix of rules for combining descriptive likelihood (Table 2.2).

- The overall likelihood that '*Ca.* L. africanus' will enter Australia by the pathways discussed in this PRA, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia are set out in Table 5.2.

**Table 5.2: Overall probability of entry, establishment and spread of '*Ca.* L. africanus' by different pathways**

<table>
<tr><th rowspan="2">Pathway</th><th colspan="3">Probability of</th><th rowspan="2">Overall probability of entry, establishment and spread</th></tr>
<tr><th>Entry</th><th>Establishment</th><th>Spread</th></tr>
<tr><td>Fruit (containing seed)</td><td>Extremely low</td><td rowspan="6">High</td><td rowspan="6">High (Very low)*</td><td>Extremely low (Extremely low)</td></tr>
<tr><td>Nursery stock (live plants)</td><td>High</td><td>High (Very low)</td></tr>
<tr><td>Budwood</td><td>Moderate</td><td>Moderate (Very low)</td></tr>
<tr><td>Fresh leaves</td><td>Extremely low</td><td>Extremely low (Extremely low)</td></tr>
<tr><td>Seed for sowing</td><td>Low</td><td>Low (Very low)</td></tr>
<tr><td>Infected psyllids</td><td>Moderate</td><td>Moderate</td></tr>
</table>

* Ratings in parenthesis are in the absence of known vectors in Australia

### 5.1.5 Consequences

The consequences of the entry, establishment and spread of '*Ca.* L. africanus' in Australia have been estimated according to the methods described in Table 2.3. The assessment of potential consequences for the bacterium is treated in combination with its psyllid vector as '*Ca.* L. species' has never been detected in the absence of a vector and economic consequences have never been assessed in isolation. The assessment is provided below:

| Criterion | Estimate and rationale |
|---|---|
| **Direct Impact** | |
| Plant life or health | **Impact score**: **E** – Significant at the regional level.<br>'*Ca*. L. africanus' is a highly destructive pathogen of citrus and is the major limiting factor for citrus production in parts of Africa (da Graça 1991; Bové 2006). Trees infected at an early age may not produce fruit, while more mature trees become unproductive soon after infection.<br>• Infected leaves generally become hard and curl outwards after getting old and occasionally have vein corking. This is followed by premature defoliation, dieback of twigs, decay of feeder and lateral roots, decline in vigour and ultimately death of the entire plant (da Graça 1991).<br>• Infected fruit are small, lopsided, remain green at the stylar end, contain aborted seed and discoloured vascular bundles, and have a bitter taste (da Graça 1991). Many fall prematurely (da Graça 1991) and potentially may taint extracted juice products, with consequent economic loss.<br>• In Africa the bacterium has killed millions of trees. Total crop loss is evident if the entire tree is infected, but it is possible that only sectors of a tree become infected and losses are small. Crop losses of 30–100% have been reported in some regions in Africa (Pretorius and Van Vuuren 2006). In the 1960s, when approximately 38% of citrus trees in South Africa were infected with HLB, production was virtually eliminated in three major citrus areas (Buitendag and von Broembsen 1993; Le Roux *et al.* 2006).<br>• The introduction of '*Ca*. L. africanus' and its vector into a climatically suitable area could result in the destruction of the citrus industry of that region, while in areas where the disease was endemic, citrus trees could live for 5–8 years (Roistacher 1996).<br>• Some Australian Rutaceae has been tested for susceptibility to '*Ca.* L. species (Halbert and Manjunath 2004). Several native and naturalised species demonstrate disease symptoms following natural infection and many more species are suspected as being hosts, but have not undergone susceptibility trials. None of the Native Australian Rutaceae considered potential hosts of '*Ca.* L. species' (Beattie and Barkley 2009) are rare or endangered (DEWHA 2009).<br>• Nurseries producing or selling Rutaceous plants can be affected significantly if '*Ca*. L. africanus' becomes established.<br>• The damage to species used as garden, amenity and household plants could be significant. Such hosts are sporadically distributed and any impact would be restricted to the local levels. |
| Other aspects of the environment | **Impact score**: **B** – Minor significance at the local level.<br>There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants<br>• In general, newly established species may affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants, degrade critical habitat or stimulate use of chemicals or biological controls. There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>  – '*Candidatus* Liberibacter africanus' is unlikely to affect the environment in these ways, as the bacterium infects and multiplies only in the Rutaceae. |
| **Indirect Impact** | |

| Criterion | Estimate and rationale |
|---|---|
| Eradication, control etc. | **Impact score**: **F** – Significant at the national level.<br>• Huanglongbing symptoms abate only temporarily when trees are injected with antibiotics (Halbert and Manjunath 2004). Consequently prevention is the only reliable control method.<br>• Once established, '*Ca*. L. africanus' is both difficult and expensive to eradicate. Eradication of the bacterium would require the removal of large numbers of native, amenity and commercial citrus plants within the vicinity of outbreaks (Beattie and Barkley 2009). Due to the large number of host plants affected, the costs of any eradication campaign are likely to be substantial.<br>• General control methods for '*Ca*. L. africanus' include certified pathogen-free nursery trees, systemic insecticides to control the psyllid vector, *T. erytreae*, and the removal of diseased trees to reduce inoculum levels (Buitendag and von Broembsen 1993; Le Roux *et al.* 2006). Implementing these measures would require a multifaceted and costly approach.<br>• While potentially able to be managed in commercial production, the presence of the bacterium will significantly increase the production costs for producers. For example, in Florida it is estimated that production costs associated with an aggressive management program for *D. citri* and '*Ca*. L. asiaticus' are 40% greater than the pre-HLB costs. Although '*Ca*. L. africanus' is considered to be less aggressive than '*Ca*. L. asiaticus', increased production costs could approach these levels (Irey *et al.* 2008).<br>• Control of psyllid vectors is the key to limiting the impact of pathogen. Foliar sprays (imidacloprid) have been used to control the psyllid vectors of the disease (Grafton-Cardwell *et al*. 2006).<br>• Considering the extreme fertility of the psyllid vector, with each female laying as many as 1000 to 2000 eggs in a matter of three weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment (Aubert 2008). |
| Domestic trade | **Impact score**: **D** – Significant at district level<br>• The presence of '*Ca*. L. africanus' in production areas may result in some domestic movement restriction for host commodities. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock, budwood and seed for sowing.<br>• Interstate restrictions on nursery stock and budwood of the Rutaceae may lead to a loss of markets, which in turn would be likely to require industry adjustment. |
| International trade | **Impact score**: **D** – Significant at district level<br>• The presence of '*Ca*. L. africanus' in production areas may limit access to some overseas markets where the pathogen is absent. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock, budwood and seed for sowing.<br>• South Africa, where this bacterium occurs, still exports fruit to Europe and the USA. |
| Environmental and non-commercial | **Impact score**: **C** – Significant at the local level.<br>• Large scale removal of native and amenity host plants would have significant effects on the environment. Broad-scale chemical treatments directed against known insect vectors may also have some impacts on native insects.<br>• Historically, the introduction of invasive agricultural pests has initiated control measures to avoid lost production. Consumer preferences for unblemished, high quality produce encourage the use of pesticides, while at the same time, negative public opinion regarding use of pesticides on fruits and vegetables is a market concern (Bunn *et al*. 1990). Therefore, the establishment of any new pests of fruits and vegetables destined for fresh markets is likely to stimulate greater use of either chemical or biological controls to ensure market access. |

Based on the decision rules described in Table 2.4, where the consequences of a pest with respect to one or more criteria are '**F**', the overall consequences are considered to be **HIGH**.

### 5.1.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for '*Ca*. L. africanus' is summarised in Table 5.3.

**Table 5.3: Unrestricted risk estimates of '*Ca*. L. africanus' for different pathways**

| Pathway | Overall probability of entry, establishment and spread | Consequences | Unrestricted risk |
|---|---|---|---|
| Fruit (containing seed) | Extremely low (Extremely low) | High | Very low (Very low) |
| Nursery stock (live plants) | High (Very low) | | High (Low) |
| Budwood | Moderate (Very low) | | High (Low) |
| Fresh leaves | Extremely low (Extremely low) | | Very low (Very low) |
| Seed for sowing | Low (Very low) | | Moderate (Low) |
| Infected psyllids | Moderate | | High |

* Ratings in parenthesis are in the absence of known vectors in Australia

The unrestricted risk for '*Ca*. L. africanus' has been assessed as 'high–low' for nursery stock and budwood, 'moderate–low' for seed for sowing and 'high' for psyllid vectors, which are all above Australia's ALOP. Therefore, specific risk management measures are required for '*Ca*. L. africanus' for nursery stock, budwood, seed for sowing and psyllid vectors. The unrestricted risk for '*Ca*. L. africanus' has been assessed 'very low' for fruit and fresh leaves, which is below Australia's ALOP. Therefore, no management measures for these pathways are required.

## 5.2 '*Candidatus* Liberibacter americanus'

Huanglongbing symptoms were found on sweet orange trees in São Paulo State, Brazil in 2004, and the casual organism was identified as a new species by PCR and named '*Ca.* L. americanus' in 2005 (Teixeira *et al*. 2005a, 2005b). This was the first reported case of '*Ca.* L. species' from the American continent. Although this bacterium was first described in 2005, it is estimated that it was present there 9–10 years before the initial detection (Lopes *et al*. 2008). Both '*Ca*. L. americanus' and '*Ca*. L. asiaticus' were discovered at the same location in two orchards in São Paulo State, Brazil, and are spreading from that point (Lopes *et al*. 2008; Lopes *et al*. 2009a). Since its discovery, '*Ca*. L. americanus' has been the most prevalent species in citrus (Gottwald *et al*. 2007), and appears to have had sufficient time to move freely from citrus to orange jasmine, without any of the limitations provided by removal of symptomatic trees or applications of insecticides—practices currently adopted in the management of HLB in citrus orchards (Lopes *et al*. 2008). The rapid spread of the pathogen in Brazil has been assisted by its vector, *Diaphorina citri*, which has been present since about 1940 (Halbert and Núñez 2004).

'*Candidatus* Liberibacter americanus' differs from '*Ca.* L. asiaticus' in graft transmission efficiency and its ability to multiply in citrus (Lopes *et al*. 2008). In the last four years, a disproportional increase in the incidence of '*Ca*. L. asiaticus' has been observed in citrus, suggesting that '*Ca.* L. americanus' is a comparatively poor competitor when in mixed species populations. Initially, '*Ca*. L. americanus' was more prevalent in São Paulo State, Brazil; however, '*Ca*. L. asiaticus' is now more prevalent (Lopes *et al*. 2009b). The increasing incidence of '*Ca*. L. asiaticus' suggests the americanus species is not a good competitor, and may exist elsewhere at very low incidences (da Graça 2008).

### 5.2.1 Probability of entry

### Pathway 1—Fruit (containing seed)

Seedless fruit poses equal or less risk than fruit containing seed. Therefore, only fruit containing seed has been considered in the pathway analysis for '*Candidatus* Liberibacter species' as the analysis is also relevant to seedless fruit.

**Probability of importation**

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia with the trade in fresh fruit (containing seeds) for consumption from countries where the pathogen is present is **VERY LOW**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter americanus' is associated with cultivated and ornamental Rutaceous host plants (Teixeira *et al*. 2005a, 2005b) and infects both fruits as well as trees (Lopes *et al*. 2007), and is therefore associated with the pathway.
- Trees that become infected with '*Ca*. L. species' in the nursery or before reaching fruit bearing age tend to have new growth that becomes systemically infected and the trees fail to produce fruit (Gottwald *et al*. 2007).
- Fruit sourced from symptomatic plants may contain the bacterium. Infected fruits are small and lopsided, exhibit strong colour inversion, seed abortion and have brown/orange stained vascular bundles (Bové 2006). There is excessive fruit drop in HLB infected trees, while fruits that remain on the tree, especially sweet oranges, can have a mottled appearance (Gottwald *et al*. 2007).
- Consumer demand for high quality citrus fruit, including the appearance and shape of the fruit, have been important in raising citrus fruit standards internationally (UNCTAD 2006). The grading of citrus fruit to meet quality standards has been defined by the international standard setting body, Codex Alimentarius. To meet internationally agreed quality standards (Codex 2005), symptomatic fruits from commercial orchards are likely to be removed during grading operations.
- Based on PCR testing of fruit parts, the peduncle, columella and seed coat from infected trees were found to contain the bacterium (Tatineni *et al*. 2008). However, the bacterium was not detected in the endosperm and embryo of seed from infected plants (Tatineni *et al*. 2008).
- The bacterium can also be retained in floral parts (petals, pistils and stamens) and could advance to young fruit during fruit development (Tatineni *et al*. 2008). For example, asymptomatic fruits sourced from symptomatic branches were considered to be in the early stages of colonization as '*Ca.* L. species' were only detected in the peduncle and central axis (Li *et al*. 2009). The bacterium was not detected in the pericarp or mesocarp, endocarp or locular membranes of the pre-symptomatic fruits (Li *et* al. 2009).
- The peduncle of fruit harvested from symptomatic trees is known to carry high concentrations of the bacterium (Tatineni *et al*. 2008). High levels of peduncle infection are likely to be associated with symptomatic fruit.

- Generally the bacterium is detected only in symptomatic fruits. Fruit is known to harbour lower concentrations of the bacterium (Floyd and Krass 2006). For example, '*Ca.* L. species' were detected in symptomatic fruits, but the population levels were 1000-fold less than the populations in the midribs, leaf blades, bark and roots (Li *et al*. 2009).
- The detection of '*Ca*. L. species' in fruit harvested from asymptomatic branches has not been recorded.
- The distribution of '*Ca.* L. species' within the host tissues is variable (Tatineni *et al*. 2008), with the level of infection in symptomatic fruit being comparatively low (Li *et al*. 2009). Based on this knowledge, the level and incidence of bacterial infection of asymptomatic fruit, particularly from healthy trees, is likely to be low.

***Ability of the pest to survive transport and storage***

- Fruit destined for Australia would be shipped in refrigerated containers maintained at 4–6 ºC. '*Candidatus* Liberibacter americanus' has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Similarities in climatic tolerances suggest that, like '*Ca.* L. africanus', storage conditions are unlikely to have any impact on the survival of '*Ca.* L. americanus' in imported fruit.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium cannot be eliminated (Floyd and Krass 2006). Pruning of infected trees is ineffective to control '*Ca*. L. americanus' in Brazil as the bacteria is systemic and remains in the roots (Lopes *et al*. 2007).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The abortion of most infected fruits, obvious symptoms of infection, international quality standards that exclude fruit damaged by pests and no known history of interception in fruit supports an assessment of 'very low'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed in a viable state within Australia with imported fruit (containing seed) from countries where the pathogen is present and transferred to a suitable host is **EXTREMELY LOW**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter species' are unable to move independently to a suitable host. Movement of '*Ca*. L. americanus' to a suitable host is only possible either by grafting or by psyllid vectors (Lopes and Frare 2008). Fruit and fruit parts are not suitable for grafting.
- Without grafting, the movement of '*Ca.* L. americanus' from imported fruit to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. americanus' is vectored by *Diaphorina citri* (Lopes and Frare 2008). This specific psyllid vector is not present in Australia.
- Species of psyllids other than *D. citri* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids, *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and

*Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009) are highly unlikely to transmit this bacterium.

- Although one species of *Diaphorina* (*D. tryoni*) is present in Australia, this species has not been recorded on the Rutaceae (DEWHA 2009).
- Furthermore, even if known psyllid vectors are present in Australia, they preferentially feed on new flush (Halbert and Manjunath 2004) and are unlikely to feed on fruits. Currently, there is no information on the ability of psyllid vectors to transmit '*Ca.* L. americanus' from infected host fruit to healthy host plants.
- Rutaceous plants are the natural hosts of '*Ca*. L. americanus' with all species and cultivars of citrus susceptible to infection (Bové 2006; Lopes and Frare 2008). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.

***Distribution of the imported commodity in the PRA area***

- Imported fruits will be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the fruit is likely to reach areas of host abundance. Distribution of infected fruit would facilitate the distribution of '*Ca.* L. americanus'.
- '*Candidatus* Liberibacter americanus' would need to survive transportation and storage within the PRA area. Fruit is typically stored and transported in refrigerated containers maintained at 4–6 ºC. '*Candidatus* Liberibacter americanus' has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Similarities in climatic tolerances suggest that, like '*Ca.* L. africanus', storage conditions are unlikely to have any impact on the survival of '*Ca.* L. americanus' in imported fruit.

***Risks from by-products and waste***

- Although the intended use of fresh fruit is human consumption, waste material would be generated (e.g. overripe and damaged fruit, uneaten portions). Whole or parts of the fruit may be disposed of at multiple locations throughout Australia in compost bins or amongst general household or retail waste.
- '*Candidatus* Liberibacter species' have been detected in infected fruit parts including peduncle, columella, seed coat and fruit peel (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures, that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. americanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008; Lopes *et al*. 2009a).
- A relatively high proportion of household and retail waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. species' from the household and environment, reducing the likelihood that susceptible plants will be exposed to this bacterium.
- If psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on fruit waste.
- Seeds present in fruit waste will desiccate quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, seeds may not germinate because they do not tolerate moisture loss.

- If seed germination from waste did occur, the seedlings would then be subject to ambient conditions, including variable moisture, temperature extremes (heat and frost) and plant competition, which will reduce seedling survival. Furthermore, seed to seedling transmission would be critical for the bacterium to enter the environment.
- Seed to seedling transmission has been reported based on symptoms (Tirtawidjaja 1981). However, as '*Ca.* L. species' are found only in the seed coat and not in the seed endosperm and embryo, it has been questioned how the bacterium enters the phloem of seedlings (Tatineni *et al*. 2008).
- Recent research suggests that while a small proportion of seedlings that develop from seed from infected plants are positive for the bacterium, the infection rate can decrease over time and symptom expression can be lost completely (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- In the absence of a suitable vector the bacterium is unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

The desiccation of citrus seed, the variable and likely unsuitable conditions for seedlings, transient seed transmission and inability of '*Ca.* L. americanus' to be distributed in the absence of a vector or graft transmission supports an assessment of 'extremely low'.

## Pathway 2—Nursery stock (live plants)

### Probability of importation

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia with trade in nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter americanus' has been reported in association with cultivated and ornamental Rutaceous host plants and can be associated with all vegetative parts of host plants (Bové 2006; Lopes *et al*. 2009b). Therefore nursery stock of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter species' can have a long latent period and many infected plants remain symptomless, particularly those which are newly infected (McClean and Oberholzer 1965b; Catling and Atkinson 1974; Bové 2006). It is highly likely that nursery stock from countries where this bacterium is present will contain the bacterium.
- '*Candidatus* Liberibacter species' survive within the vascular system of the host plants and are confined to the living phloem cells (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant, which other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).
- Since '*Ca*. L. species' infect phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). Symptom expression is influenced by temperature and varies with season (Lopes *et al*. 2009a). '*Candidatus* Liberibacter americanus' has similar temperature sensitivities to that of '*Ca*. L. africanus', described in Section 5.1.2 (Lopes *et al*., 2009b) and in unfavourable environments symptoms may not be expressed (Bové 2006).
- '*Candidatus* Liberibacter americanus' affects all commercial cultivars of mandarin and sweet orange in Brazil (Lopes and Frare 2008), regardless of rootstock and age of the tree (Lopes *et al*. 2009b).

- Symptoms may include leaf yellowing on a single shoot or branch, a mottled or blotchy appearance and deformed fruits that fall pre-maturely (Lopes and Frare 2008). Usually, the symptoms appear first on mature leaves close to the top of the branches and then progress throughout the entire tree (Lopes *et al*. 2007). Symptomatic nursery stock is likely to be detected on arrival in Australia.
- '*Candidatus* Liberibacter americanus' is unable to multiply efficiently in citrus plants (Lopes *et al*. 2008) and its titres in hosts are much lower than '*Ca*. L. asiaticus' titres (Lopes and *et al.* 2009b). Field observations over a period of four years found that the proportion of hosts infected by '*Ca*. L. americanus' has decreased from 98% to 20% and over the same period the proportion of hosts infected by '*Ca*. L. asiaticus' has increased from 2% to 80% (Lopes *et al.* 2009b).
- Infected nursery stock may be asymptomatic in the initial stages of infection and can be easily overlooked. It is reported that 15–20% of HLB infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- '*Candidatus* Liberibacter species' are difficult to detect because they are often present at a low concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009). In asymptomatic foliage, the bacterium can only be detected if the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008).
- Association with nursery stock can provide long term survival for the pathogen. The bacteria have a long incubation period and many infected plants remain symptomless, particularly those that are newly infected (Bové 2006). It is likely that asymptomatic plants infected by '*Ca.* L. americanus' would pass routine visual inspections and be released into Australia.

***Ability of the pest to survive transport and storage***

- Nursery stock is expected to be shipped at moderate temperatures and humidity levels to ensure nursery stock survival; conditions that are unlikely to adversely affect '*Ca*. L. americanus' that are present during shipment.
- '*Candidatus* Liberibacter species', '*Ca*. L. africanus' and '*Ca*. L. asiaticus', have been introduced into new areas by the importation of infected planting material (Bové 2006; Pretorius and Van Vuuren 2006; Gottwald *et al*. 2007).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium cannot be eliminated (Floyd and Krass 2006). Pruning of infected trees is ineffective to control '*Ca*. L. americanus' in Brazil as the bacteria is systemic and remains in the roots (Lopes *et al*. 2007).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The association of '*Ca*. L. americanus' with nursery stock, the ability for infected plants to remain asymptomatic and the likelihood that the bacterium will remain viable during transport and storage supports an assessment of 'high'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed within Australia with imported nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter americanus' arriving in Australia with nursery stock will not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host. '*Candidatus* Liberibacter americanus' is limited to phloem (Lopes *et al*. 2009b) and the infection is systemic with an uneven distribution of the pathogen within the host tissues (Huang 1979).
- Natural movement of '*Ca*. L. species' from infected nursery stock to a suitable host, in the absence of vectors, is limited or absent (Polek *et al.* 2007). The bacterium can move to a suitable host only by grafting (Aubert 2008).
- Without grafting, the transfer of '*Ca.* L. americanus' from imported nursery stock to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. americanus' is vectored by *Diaphorina citri* (Teixeira *et al*. 2005b). This specific psyllid vector is not present in Australia.
- Nursery stock is imported specifically for the purpose of propagation. Infected nursery stock is therefore likely to be planted directly into suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported nursery stock.
- Rutaceous plants are the natural hosts of '*Ca*. L. americanus' with all species and cultivars of citrus susceptible to infection (Lopes and Frare 2008). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to these hosts in the PRA area.
- There is one species of *Diaphorina* present in Australia, *D. tryoni.* However, this species has not been recorded on members of the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* have been recorded on Rutaceous hosts, where '*Ca.* L. species' are present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- '*Candidatus* Liberibacter americanus' is unable to multiply efficiently in citrus as its titre is low in the plant (Lopes *et al.* 2009a). High titres increase the chances of acquisition and transmission by insect vectors (Lopes *et al*. 2009a).
- In the absence of a vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the pathogen will fail to persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- The distribution of nursery stock would be for retail distribution to multiple destinations throughout Australia. Nursery stock would also be distributed to multiple locations for propagation. Distribution of infected nursery stock commercially through nurseries would facilitate the distribution of '*Ca*. L. americanus'.
- Infected nursery stock may be distributed to glasshouses or retail shops where the bacterium may continue multiplying within the host. Infected nursery stock is unlikely to be grown in isolation, providing greater opportunity for the bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected nursery stock to other hosts.
- '*Candidatus* Liberibacter americanus' would need to survive transportation and storage within the PRA area. Nursery stock is expected to be maintained at moderate

temperatures and humidity levels to ensure nursery stock survival, so a portion of infected nursery stock that enters the country is likely to reach areas of host abundance.

- As nursery stock may not display obvious symptoms of '*Ca.* L. americanus' infection, there is a risk that infected plant material would be used for propagation. Material from infected plants may be used for grafting and grafted rootstock is planted directly at multiple locations in Australia.
- Asymptomatic plants may also be overlooked and sold to commercial users and households.

***Risks from by-products and waste***

- Although the intended use of nursery stock is for propagation, and all imported material would be grown under ideal conditions, waste material may be generated. Whole or parts of the plants may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures, that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. americanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008; Lopes *et al*. 2009a).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if known psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread the bacterium to other hosts. However, acquisition and transmission periods for *D. citri* vary from 1–25 days from living host tissue (Halbert and Manjunath 2004).

The association of '*Ca.* L. americanus' with asymptomatic nursery stock and the likely distribution of infected plants for propagation support an assessment of 'high' for the distribution of this species in nursery stock.

## Pathway 3—Budwood

### Probability of importation

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia with trade in budwood from countries where the pathogen is present is **MODERATE**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter americanus' has been reported in association with cultivated and native Rutaceous species and can be associated with all vegetative parts of host plants (Bové 2006; Lopes *et al*. 2009b). Therefore budwood can provide a pathway for the importation of '*Ca.* L. americanus' into Australia.
- '*Candidatus* Liberibacter species' survive within the living phloem cell, in the vascular system of host plants (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant, which other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).

- Since '*Ca*. L. species' infect phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). However, the bacterium has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present in all stems of infected trees.
- Infected field trees can be identified by their foliar and fruit symptoms (Polek *et al*. 2007). Budwood is high value and a large investment is made in the health and survival of mother plants. Therefore, it is unlikely that budwood would be sourced from symptomatic trees.
- '*Candidatus* Liberibacter species' can have a long latent period and many infected plants remain symptomless, particularly those which are newly infected (McClean and Oberholzer 1965b; Catling and Atkinson 1974; Bové 2006). It is therefore likely that budwood sourced from asymptomatic trees could contain low concentrations of the bacterium (Huang *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter species' can be detected in asymptomatic hosts only when the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008). However, '*Ca.* L. species' are difficult to detect because they are often present at a low concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter americanus' was detected on scions developed from buds of symptomatic branches, but not from asymptomatic branches of the same affected trees (Lopes *et al*. 2009b).
- '*Candidatus* Liberibacter americanus' has similar temperature sensitivities to that of '*Ca*. L. africanus', described in Section 5.1.2 (Lopes *et al*., 2009b) and in unfavourable environments symptoms may not be expressed (Bové 2006).
- Infected mother plants may be asymptomatic in the initial stages of infection and can be easily overlooked when sourcing budwood. For example, it is reported that 15–20% of HLB infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- Once budwood has been harvested from infected trees the bacterium does not produce visible symptoms on budwood and may escape detection during routine visual inspection. Association with budwood can provide long term survival for the bacterium.

***Ability of the pest to survive transport and storage***

- Budwood is expected to be shipped at moderate temperatures and humidity levels to ensure survival; conditions that are unlikely to adversely affect any '*Ca*. L. americanus' present during shipment.
- '*Candidatus* Liberibacter species', '*Ca*. L. africanus' and '*Ca*. L. asiaticus', have been introduced into new areas by the importation of infected planting material (Bové 2006; Pretorius and Van Vuuren 2006; Gottwald *et al*. 2007).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium cannot be eliminated (Floyd and Krass 2006). Pruning of infected trees is ineffective to control '*Ca*. L. americanus' in Brazil as the bacteria is systemic and remains in the roots (Lopes *et al*. 2007).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

Although '*Candidatus* Liberibacter americanus' is associated with budwood and the bacterium would remain viable during transport and storage, the bacterium is unevenly distributed within host trees and may not be present in budwood sourced from infected asymptomatic hosts. Therefore, an assessment of 'moderate' for the importation of this species in budwood is allocated.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed within Australia with imported budwood from countries where the pathogen is present is **HIGH**.

#### *Ability of the pest to move from the pathway to a suitable host*

- '*Candidatus* Liberibacter americanus' arriving in Australia with imported budwood will not need to move from the import pathway to a suitable host as the bacterium is already within a suitable host (Lopes and Frare 2008; Lopes *et al*. 2009b).
- '*Candidatus* Liberibacter americanus' is unable to move independently from imported budwood to a suitable host. Movement of '*Ca*. L. americanus' to a suitable host is only possible either by grafting or by psyllid vectors (Lopes and Frare 2008; Lopes *et al*. 2009b).
- Budwood is imported specifically for the purpose of grafting onto local rootstock, which will then be planted directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the grafted rootstock. Therefore the bacterium will move from imported budwood to a suitable host by grafting.
- Graft transmission has been demonstrated for '*Ca*. L. americanus' (Lopes *et al*. 2009b). '*Candidatus* Liberibacter americanus' is not efficiently graft-transmissible from infected to healthy tissue (Lopes *et al*. 2009b) and it is also unable to multiply efficiently in citrus plants (Lopes *et al*. 2008). Therefore, the grafting technique and low bacterial titres will affect symptom expression and could prevent early detection.
- Rutaceous plants are the natural hosts of '*Ca*. L. americanus', with all species and cultivars of citrus susceptible to infection (Lopes and Frare 2008). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. americanus' is vectored by *Diaphorina citri* (Teixeira *et al*. 2005b). This specific psyllid vector is not present in Australia.
- Species of psyllids other than *D. citri* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta* known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- A single species of *Diaphorina* (*D. tryoni*) is present in Australia, but it has not been recorded on the Rutaceae (DEWHA 2009).
- In the absence of a suitable vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

#### *Distribution of the imported commodity in the PRA area*

- The imported budwood would be distributed to multiple destinations throughout Australia for propagation. Distribution of infected budwood commercially would facilitate the distribution of '*Ca*. L. americanus'.

- Plants grafted with imported infected budwood may be distributed to greenhouses or retail shops where the bacterium may continue multiplying within the host. Grafted plants are unlikely to be grown in isolation, providing greater opportunity for this bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to other hosts.
- Asymptomatic plants resulting from grafting with imported budwood may also be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter americanus' would need to survive transportation and storage within the PRA area. Grafted plants are expected to be maintained at moderate temperatures and humidity levels to ensure their survival, so a high portion of infected plants are likely to reach areas of host abundance.

***Risks from by-products and waste***

- Although the intended use of budwood is for propagation, and all imported material is likely to be used, waste material may be generated. Whole or parts of the budwood may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing the bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. americanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008; Lopes *et al*. 2009a).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However, acquisition and transmission periods for *D. citri* vary from 1–25 days from living host tissue (Halbert and Manjunath 2004).

The association of '*Ca*. L. americanus' with budwood and the likely distribution of infected budwood and grafted plants support an assessment of 'high' for the distribution of this species in budwood.

## Pathway 4—Fresh leaves

### Probability of importation

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia with the trade in fresh leaves from countries where the pathogen is present is **MODERATE**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter species' can be associated with all vegetative parts of host plants including foliage (Tatineni *et al.* 2008), so fresh leaves of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter americanus' survives within the phloem of the vascular system in host plants (Bové 2006). Although the bacterium infects phloem, the infection has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present in the leaves of infected trees.

- Symptoms of leaves infected by '*Ca.* L. species' include a mottled or blotchy appearance at the initial stage of development and vein corking, which is typified by bright yellow leaf veins that are raised and have a corky appearance (Spann *et al*. 2008). Symptomatic leaves would most likely be detected on arrival in Australia.
- Symptom expression is influenced by temperature and varies with season. '*Candidatus* Liberibacter americanus' has similar temperature sensitivities to that of '*Ca*. L. africanus' (Lopes *et al*. 2009a) and in unfavourable environments symptoms may not be expressed (Bové 2006).
- Infected plants may be asymptomatic in the initial stages of infection and could be easily overlooked when harvesting leaves for export.
- Symptomatic leaves contain higher concentrations of the bacterium than asymptomatic leaves (Bové 2006; Trivedi *et al*. 2009). Higher concentrations of '*Ca*. L. americanus' have been reported in symptomatic leaves in Brazil (Teixeira *et al*. 2008).
- Teixeira *et al.* (2008) extrapolated their experimental results and estimated that the percentage '*Ca.* L. species' infection in asymptomatic leaves was 17%. The bacterial titre in the asymptomatic leaves varied from low to high concentrations.
- The bacteria have a long incubation period and many infected plants remain symptomless, particularly those which are newly infected (Bové 2006). It is likely that asymptomatic leaves infected by '*Ca.* L. americanus' would pass routine visual inspections.

***Ability of the pest to survive transport and storage***

- Fresh leaves destined for Australia would be air freighted and, during transportation and storage, kept at low temperatures and high humidity to ensure the material remains fresh. '*Candidatus* Liberibacter americanus' has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Therefore, low temperatures and high humidity are unlikely to have any impact on the survival of '*Ca.* L. americanus' bacteria in imported fresh leaves.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium cannot be eliminated (Floyd and Krass 2006). Pruning of infected trees is ineffective to control '*Ca*. L. americanus' in Brazil as the bacteria is systemic and remains in the roots (Lopes *et al*. 2007).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

'*Candidatus* Liberibacter americanus' is associated with fresh leaves, the foliage of infected plants may remain asymptomatic and the bacterium is likely to remain viable during transport and storage. However, the bacterium is unevenly distributed within host trees and may not be present in the leaves sourced from infected hosts. Therefore an assessment of 'moderate' for the importation of this species in fresh leaves is allocated.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed within Australia in a viable state with imported fresh leaves from countries where the pathogen is present and transferred to a suitable host is **EXTREMELY LOW**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter americanus' is unable to move independently from imported fresh leaves to a suitable host. The bacterium is able to move to a suitable host only by grafting or by psyllid vectors (Lopes and Frare 2008). Cut leaves are not suitable for grafting.
- Without grafting, the transfer of '*Ca.* L. africanus' from imported fresh leaves to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. americanus' is vectored by *Diaphorina citri* (Lopes and Frare 2008). This specific psyllid vector is not present in Australia.
- There is one species of *Diaphorina* present in Australia, *D. tryoni.* However, this species has not been recorded on members of the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta* known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- Furthermore, even if known psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on cut leaves.

***Distribution of the imported commodity in the PRA area***

- Asymptomatic leaves will be distributed for all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption (e.g. curry leaves).
- Fresh leaves may be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the produce is likely to reach areas of host abundance.
- '*Candidatus* Liberibacter americanus' would need to survive transportation and storage within the PRA area. Fresh leaves would be kept at low temperatures and high humidity to ensure the material remains fresh. Thus, transport and storage conditions are unlikely to have any impact on the survival of '*Ca.* L. americanus' in fresh leaves distributed for retail or market sale.

***Risks from by-products and waste***

- Although the intended use of fresh leaves includes all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption, waste material would be generated. Fresh leaves may be disposed of at multiple locations throughout Australia in compost bins or amongst general waste.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem limited bacteria. For example, composting waste material is likely to generate high temperatures, that can be lethal to a range of pathogens Noble and Roberts 2004), and '*Ca*. L. americanus' is heat-sensitive to temperatures above 32 °C (Bové *et al*. 2008; Lopes *et al*. 2009a).
- A relatively high proportion of household and retail waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. species' from the household and environment, reducing the likelihood that susceptible plants will be exposed to this bacterium.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However,

acquisition and transmission periods for *D. citri* vary from 1–25 days (Halbert and Manjunath 2004).

Although '*Ca.* L. americanus' is associated with fresh leaves, the bacterium is unable to disperse unassisted, is unlikely to be transmitted by a vector even if the known vector is present in Australia (as vectors prefer to feed on fresh foliage) and the pathway is unsuitable for graft transmission. Therefore an assessment of 'extremely low' is given for the distribution of this species in fresh leaves.

## Pathway 5—Seed for sowing

### Probability of importation

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia with trade in seed of Rutaceous species for sowing from countries where the pathogen is present is **LOW**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter species' have been detected in citrus seed coat but not in the seed endosperm and embryo (Tatineni *et al*. 2008; Li *et al*. 2009). Therefore this bacterium is associated with the pathway.
- Seeds from '*Ca.* L. species' affected trees often show various degrees of abortion (Bové 2006), probably related to the presence of the pathogens in the seed coats.
- Based on research into seed to seedling transmission at low levels (Benyon *et al*. 2008; Graham *et al*. 2008; Hartung *et al*. 2008; Shatters 2008) the USA banned the importation of seed for sowing from species of known host genera in the Rutaceae from countries where citrus infecting '*Ca.* L. species' are present (APHIS 2008b).
- A proportion of fruit on infected trees is aborted and the remaining fruit has a high rate of aborted seed (Halbert and Manjunath 2004). Un-aborted seed in infected fruits are characteristically small and brownish/black in colour (Bové 2006). Such seeds may be rejected due to quality issues.
- The distribution of '*Ca.* L. species' within the host tissues is variable, with the level of infection in symptomatic fruit being comparatively low (Tatineni *et al*. 2008; Li *et al*. 2009).
- Asymptomatic fruit, harvested from symptomatic branches, have tested positive for the bacteria (Li *et al*. 2009). The detection of '*Ca*. L. species' in fruit harvested from asymptomatic branches has not been recorded. Based on this knowledge, the level and incidence of bacterial infection of seed from asymptomatic fruit, particularly from healthy trees, is likely to be low.

#### *Ability of the pest to survive transport and storage*

- Seed would be shipped in refrigerated containers maintained at 3–7 ºC (Willits and Newcomb Inc 2004). '*Candidatus* Liberibacter americanus' has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Similarities in climatic tolerances suggest that, like '*Ca.* L. africanus', storage conditions are unlikely to have any impact on the survival of '*Ca.* L. americanus' in imported seed.
- Citrus seed is highly perishable and sowing should occur as soon as possible following importation. Temperature extremes and exposure to direct sunlight may be fatal to citrus seeds (Willits and Newcomb Inc 2004). Thus, the length of time taken to transport citrus seed from the country of origin, and the time it spends in storage prior to export, may lower its viability and that of any associated bacterium.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium cannot be eliminated (Floyd and Krass 2006). Pruning of infected trees is ineffective to control '*Ca*. L. americanus' in Brazil as the bacteria is systemic and remains in the roots (Lopes *et al*. 2007).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The lack of fruit produced by infected trees, the high rate of aborted seeds and the discolouration of the seeds supports an assessment of 'low' for the importation of '*Ca*. L. americanus' in seed.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed within Australia with imported seed from countries where the pathogen is present is **MODERATE**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter americanus' arriving in Australia with seed may not need to move from the import pathway to a suitable host as the pathogen is already present within a suitable host. '*Candidatus* Liberibacter species' can be detected at a high level in the seed coats (Tatineni *et al*. 2008).
- Unlike seed imported in fruit, seed for sowing is imported specifically for the purpose of propagation and can be a significant investment for importers. Infected seed is therefore likely to be sown directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported seed.
- Although, '*Ca.* L. americanus' is unable to move independently from imported seed to a suitable host, the bacterium is able to move from the imported seed to the resultant seedling.
- Seed to seedling transmission has been reported when seed sourced from infected plants were grown (Tirtawidjaja 1981; Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008). However, the infection rate can decrease over time and symptom expression can be lost completely (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- Recent experimental studies have confirmed the seed to seedling transmission of '*Ca*. L. species' (Benyon *et al*. 2009). However, over a three year period, the bacterial titre remained at very low levels and most, if not all, of the seedlings did not develop typical HLB symptoms (Benyon *et al*. 2009).
- There is no report of seed transmission in *Murraya* species or any other citrus relative. However, seed transmission (as high as 53%) of a related species, '*Ca*. L. asiaticus', was recently reported experimentally for dodder and periwinkle (Zhou *et al*. 2008b).
- If an infected seedling does establish, insect vectors would be required to move the bacterium to new hosts. In the absence of a vector, the bacterium is unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).
- Rutaceous plants are the natural hosts of '*Ca*. L. americanus' with all species and cultivars of citrus susceptible to infection (Lopes and Frare 2008; Lopes *et al*. 2007). These hosts are widespread in cities, towns and horticultural production areas

throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.

***Distribution of the imported commodity in the PRA area***

- The distribution of seed for sowing would be for commercial and retail distribution to multiple destinations throughout Australia. Seed for sowing would also be distributed throughout the PRA area for propagation.
- Seeds would be imported to introduce new genetic material to commercial citrus production. Imported citrus seeds are likely to be distributed to suitable areas where citrus is grown.
- Distribution of infected seed commercially through nurseries would facilitate the distribution of '*Ca*. L. americanus'. Asymptomatic plants that develop from infected seed may be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter americanus' would need to survive transportation and storage within the PRA area. '*Candidatus* Liberibacter americanus' has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006). Similarities in climatic tolerances suggest that, like '*Ca.* L. africanus', storage conditions are unlikely to have any impact on the survival of '*Ca.* L. americanus' in imported seed.

***Risks from by-products and waste***

- The intended use of seed is for propagation, and all imported seed would be grown under ideal conditions. Seed that does not survive transport and storage may shrivel and discolour, and may not be used for sowing. Therefore, waste material may be generated. As the bacterium can infect the entire plant (Tatineni *et al*. 2008) any such material that is discarded may contain the bacterium.
- Seeds of most *Citrus* species desiccate quite quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, discarded seed may not germinate because they do not tolerate moisture loss.
- If seed does germinate and seed transmission does occur from these discarded seeds, an infected plant will establish; however, the infection rate can decrease over time (Graham *et al*. 2008).
- The transfer of '*Ca.* L. americanus' from an infected seedling established at waste depot or in the backyard to a host would require a vector. The only known vector is not present in Australia.
- Since '*Ca*. L. species' are spread only by a vector or grafting, it is highly likely that an infected plant that develops in an area where the psyllid vector is not known to occur will represent an isolated infection (Floyd and Krass 2006).

Although, '*Ca.* L. species' are associated with seeds at variable rates, the low level of seed to seedling transmission and loss of symptoms over time, support an assessment of 'moderate' for the distribution of '*Ca.* L. americanus' in seeds.

## Pathway 6—Infected Psyllid

### Probability of importation

The likelihood that '*Candidatus* Liberibacter americanus' will arrive in Australia in an infective psyllid vector (*Diaphorina citri*) from countries where the pathogen is present is **MODERATE**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter americanus' is naturally associated with *D. citri* (Teixeira *et* al. 2005c; Lopes and Frare 2008). Therefore this bacterium is associated with the pathway.
- *Diaphorina citri* feeding on infected host plants may acquire the bacterium (Lopes and Frare 2008). Once the psyllid has acquired the bacterium, based on the persistent survival of '*Ca*. L. species' in *D. citri* (Bové 2006), the vector is likely to maintain the ability to spread the bacterium throughout its life.
- Large numbers of '*Ca.* L. americanus' can be observed in psyllids (Teixeira *et al*. 2005b), which may suggest that the bacterium multiplies inside the insect (da Graça 2008).
- Infections of the psyllid by the bacterium, multiplication of the bacterium in the psyllid and transovarial transmission are important components of interaction between '*Ca*. L. species' and their vectors (Bové 2006; Rogers *et al* 2008).
- There is no information in the literature regarding the detection, or transovarial transmission, of '*Ca*. L. americanus' in different life stages of *D. citri*. '*Candidatus* Liberibacter asiaticus', which is also vectored by *D. citri*, has been detected in the second, third, fourth and fifth instar nymphs and adults of *D. citri* (Hung *et al*. 2004). '*Candidatus* Liberibacter africanus' can infect all life stages of its psyllid vector *Trioza erytreae* through transovarial infection, and direct infection of late stage nymphs and adults through feeding on infected plants (Van den Berg *et al*. 1991–1992). Based on this information, both the juvenile and adult stages of *D. citri* have the potential to be infected with '*Ca*. L. americanus'.
- '*Candidatus* Liberibacter americanus' is unable to multiply efficiently in citrus plants (Lopes *et al*. 2008) and its titres in hosts are much lower than '*Ca*. L. asiaticus' titres. This may result in lower vector acquisition and transmission rates (Lopes and *et al.* 2009b). Higher titres increase the chances for pathogen acquisition and transmission by the insect vector (Lopes *et* al. 2009a).
- The proportion of infected psyllids in a given psyllid population is variable. Studies conducted in Brazil indicated that the number of adult *D. citri* carrying '*Ca*. L. americanus' is relatively small in relation to the total adult population found on trees (Teixeira *et al.* 2005b). Adult psyllids collected from a tree infected with '*Ca.* L. americanus', showed six out of 22 samples were infected with the bacteria, giving a maximum psyllid infection rate of 27%. However, samples were lots of 10 psyllids and thus the actual infection rate is probably lower than 27% (Teixeira *et al.* 2005b).
- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004; Aubert 1987b). It would be possible for infected psyllids to enter Australia via trade in commodities, such as fresh fruit, fresh leaves, and budwood and nursery stock of suitable host species (Refer to Chapter 5.4).
- Leaf material or trash in imported fruit is a significant risk for the entry of all life stages of *D. citri* as eggs are laid on the leaves (Grafton-Cardwell *et al*. 2006) and nymphs and adults feed under the surfaces of leaves (Shivankar *et al*. 2000; CABI 2007).

***Ability of the pest to survive transport and storage***

- Once infected with '*Ca.* L. species', psyllids remain infective for life (Gottwald *et al.* 2007). '*Candidatus* Liberibacter americanus' is therefore likely to survive for long periods in infective psyllids provided the conditions are suitable for the survival of the psyllids.

- Infected psyllids are likely to enter Australia via trade in fresh fruit, fresh leaves, nursery stock and budwood of suitable host species (Refer to Chapter 5.4) and must survive the transport and storage conditions for these commodities in order to vector '*Ca.* L. americanus'.
- Adult *D. citri* are able to survive up to 52.5–94.5 hours without feeding if suitable foliage is not available (McFarland and Hoy 2001).
- Infected psyllids associated with fresh leaves or nursery stock have the potential to continue feeding during transport and storage. Adult *D. citri* may survive for 8–9 months over winter provided suitable foliage is available (Yang *et al*. 2006).
- The threshold temperature for nymphal development is 10–11 °C (Liu and Tsai 2000) and the commodities they are likely to be in association with will be shipped at low (4–6 °C for fruit) to moderate temperatures.
- Transport temperatures are not lethal to *D. citri* as the psyllid is able to survive low temperatures (Hall 2008b). *Diaphorina citri* tolerate low ambient temperatures (e.g. mid winter average daily temperatures between 5.4–6.9 °C, with minimums from -5.3 to -7.5 °C (Xie *et al.* 1988). These conditions are unlikely to have any impact on the survival of '*Ca*. L. americanus' within the psyllid.
- There is no reason to suspect that the duration of transport and storage would lower the viability of '*Ca*. L. americanus' in *D. citri*.
- There have been 170 interceptions of live *D. citri* at ports in the USA, on plant material with an Asian origin, from 1985–2003 (Halbert and Manjunath 2004; Sullivan and Zink 2007). There are an additional 73 records of interceptions of live *Diaphorina* species on Rutaceous plants from Asia (Halbert and Manjunath 2004). This indicates that *D. citri* would survive during transport, particularly on hosts transported by air where transit times will be short.
- Live *D. citri* have been repeatedly intercepted on citrus fruit from the Bahamas and shipped to Florida for processing (Halbert and Núñez 2004; Sullivan and Zink 2007). In addition, *D. citri* has been intercepted from Belize on citrus in baggage at Houston Airport, Texas (Halbert and Núñez 2004). It has also been detected on citrus fruit from Mexico entering Texas (Halbert and Núñez 2004).
- Live *D. citri* were intercepted in shipments of fresh curry leaves (*M. koenigii*) from Hawaii to California (Wilkinson 2007; Filippini 2008). Recently, about 100 live nymphs and adults were detected on fresh curry leaves in a package from Texas to California (CDFA 2009; The Packer 2009).
- Live nymphs and adults of *D. citri* were detected on fresh curry leaves in unaccompanied baggage from India to California in July 2009. Subsequent testing confirmed the presence of the HLB pathogen in one of the insects (CDFA 2009; Associated Press 2009).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated (Floyd and Krass 2006).
- Insecticides, neem oil, petroleum spray oil and biological control agents may be used in orchard management of psyllids (Aubert and Quilici 1984; Xu *et al*. 1991; Chakravarthi *et al*. 1998; Rae *et al*. 1997). These control methods will likely reduce, but not necessarily eliminate, infected *D. citri*. The methods will have no impact on the level of '*Ca.* L. americanus' infection in surviving psyllids.

The ability of '*Ca.* L. americanus' to infect *Diaphorina citri*, the association of the psyllid with host plants, the variable number of infected psyllids and the likelihood of the bacterium remaining viable within the psyllid for long periods during transport and storage supports an assessment of 'moderate'.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter americanus' will be distributed within Australia with infected psyllid to suitable hosts from countries where the pathogen is present is **HIGH**.

### *Ability of the pest to move from the pathway to a suitable host*

- '*Candidatus* Liberibacter americanus' arriving in Australia with infected *D. citri* may continue to multiply inside the psyllid vector (da Graça 2008).
- For '*Ca.* L. americanus' to be transferred from the psyllid to a plant host, infected psyllids would need to complete their development to the adult stage (if they are immature), find a host to feed on and successfully transmit the bacterium to the host.
- Sessile nymphs infected with '*Ca.* L. species' are known to retain the bacterium on maturing to adults (Halbert and Manjunath 2004).
- Immature stages of the psyllid must have suitable food sources to allow them to survive until they reach adulthood. Due to the relatively long developmental time—48 days in winter from egg to adult (Halbert and Manjunath 2004; EPPO 2009), there is likely to be some mortality of eggs and nymphs.
- Adults of *D. citri* have been recorded to regularly fly distances of 8–60 m (Hall 2008a), and to travel 0.5–1 km in wind drifts (Barkley and Beattie 2008), and are able to survive up to 52.5–94.5 hours without feeding if suitable foliage is not available (McFarland and Hoy 2001). Therefore *D. citri* carrying '*Ca*. L. americanus' is capable of locating potential host plants to transmit the bacterium.
- Rutaceous plants are the natural hosts of '*Ca*. L. americanus' with all species and cultivars of citrus susceptible to infection (Lopes and Frare 2008; Lopes *et al*. 2007). *Diaphorina citri* feed and reproduce on the Rutaceae, including cultivated *Citrus* species and Australian naturalised/native species *Murraya paniculata* and C*itrus australasica* (Halbert and Manjunath 2004). There is a significant overlap between the host range of the bacterium and the psyllid suggesting the psyllid is likely to distribute the bacterium to a suitable host.
- Suitable hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- Once a suitable host has been found, infected adult psyllids are able to transmit the pathogen (Gottwald *et al.* 2007). However, the rate of transmission has not been recorded directly.

### *Distribution of the imported commodity in the PRA area*

- Psyllids carrying '*Ca.* L. americanus' imported into Australia on fruit, fresh leaves, budwood and/or nursery stock of host plants may be distributed throughout the PRA with these commodities as the commodities are imported for wholesale and retail sale to multiple locations.
- Infected *D. citri* would need to survive transportation and storage within the PRA area. The threshold temperature for nymphal development is 10–11 °C (Liu and Tsai 2000) and '*Ca.* L. americanus' is likely to survive lower temperatures since the bacterium has similar environmental tolerances to '*Ca.* L. africanus' (Lopes *et al*. 2009a), which survives in cool regions, at high elevations (Schwarz and Green 1972; Bové 2006).

- *Diaphorina citri* survives in a wide range of temperatures extremes, from 45 °C in Saudi Arabia to -7 °C or -8 °C in subtropical China (Aubert 1988, 1990) and is therefore likely to survive transport and storage temperatures. It is considered unlikely that transport and storage conditions would reduce or eliminate '*Ca*. L. americanus' within the psyllid.

***Risks from by-products and waste***

- Infected psyllids are likely to be distributed throughout the PRA area with fresh fruit, fresh leaves, and budwood and/or nursery stock of Rutaceous hosts. Although the intended use of these commodities is for human consumption (fruit, fresh leaves) and propagation (nursery stock and budwood), waste material would be generated. Whole or parts of the imported fruit, fresh leaves, budwood or nursery stock infested or contaminated with life stages of the psyllid may be disposed of at multiple locations throughout Australia in compost bins or amongst general household waste.
- Infected psyllids may survive on waste material for a short time before dispersing to suitable hosts. *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to fly significant distances autonomously (Barkley and Beattie 2008; Hall 2008a).

The association of '*Ca*. L. americanus' with *Diaphorina citri*, the ability for infected psyllids to disperse both independently and through the movement of infested host commodities, the host range overlap of the two species, and the presence of multiple hosts within the PRA area support an assessment of 'high' for the distribution of '*Ca*. L. americanus' in *Diaphorina citri*.

### Overall probability of entry (importation x distribution)

The overall probability of entry of '*Ca*. L. americanus' is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2). The overall probability of entry for the six pathways being assessed in this PRA is set out in Table 5.4.

**Table 5.4: Overall probability of entry of '*Ca*. L. americanus' on different pathways**

| Pathway | Probability of importation | Probability of distribution | Overall probability of entry |
|---|---|---|---|
| Fruit (containing seed) | Very low | Extremely low | Extremely low |
| Nursery stock (live plants) | High | High | High |
| Budwood | Moderate | High | Moderate |
| Fresh leaves | Moderate | Extremely low | Extremely low |
| Seed for sowing | Low | Moderate | Low |
| Infected psyllids | Moderate | High | Moderate |

## 5.2.2 Probability of establishment

The likelihood that '*Candidatus* Liberibacter americanus' will establish within Australia, based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is **HIGH**.

***Availability of suitable hosts, alternative hosts and vectors in the PRA area***

- Rutaceous plants are the natural hosts of '*Ca*. L. americanus' with all species and cultivars of citrus susceptible to infection (Lopes and Frare 2008; Lopes *et al*. 2007). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

- '*Candidatus* Liberibacter species' are vector specific and '*Ca*. L. americanus' is naturally vectored by *Diaphorina citri* (Teixeira *et al.* 2005b), which could provide a means for the establishment of this bacterium if introduced into Australia.
- *Diaphorina citri* was detected in the Northern Territory in 1915, but was eliminated during the 1918–1922 citrus canker eradication programs, and there have been no detections of this pest since (Bellis *et al.* 2005). There has never been a detection of HLB in Australia (Bellis *et al.* 2005).
- Although one species of *Diaphorina* (*D. tryoni*) is present in Australia, this species has not been recorded on the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta* known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- In the absence of a vector, the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Suitability of the environment***

- '*Candidatus* Liberibacter americanus' has established successfully in the state of Sao Paulo, Brazil (Teixeira *et al*. 2008). The climatic regions across this range include temperate areas and there are similar climatic regions in parts of Australia that would be suitable for the establishment of '*Ca*. L. americanus' (Peel *et al.* 2007).
- '*Candidatus* Liberibacter americanus' is heat sensitive (Lopes *at al*. 2009a). This sensitivity explains the absence of '*Ca*. L. americanus' in the hot northwest region of São Paulo State (Lopes *et al*. 2009a). The optimum temperature for establishment and expressing symptoms for '*Ca*. L. americanus' is 22–24 °C, while temperatures of 32 °C or above are detrimental to this bacterium (Lopes *at al*. 2009b).
- '*Candidatus* Liberibacter americanus' is therefore more likely to establish in the temperate southern regions of Australia and less likely to establish in the more tropical northern regions.
- '*Candidatus* Liberibacter americanus' and its vector, *D. citri* have different environmental preferences. The bacterium is heat-sensitive whereas the vector is heat-tolerant. This environmental preference indicates that only the southern regions of Australia would have climates suitable for '*Ca*. L. americanus' whereas *D. citri* will survive in a wider range of climates in Australia.

***The reproductive strategy and survival of the pest***

- '*Candidatus* Liberibacter americanus' multiplies and survives in the phloem of infected host plants (Teixeira *et al*. 2005a) and has been detected in *Diaphorina citri* (Teixeira *et al*. 2005b).
- '*Candidatus* Liberibacter americanus' is reliant on infected plants for survival and will survive as long as the infected plant survives. Symptom development may be delayed for several months, or as long as two to three years after initial infection (Capoor *et al.* 1974; Hung *et al*. 2001; Gottwald *et al*. 2007). It is reported that infected plants show severe symptoms after one to five years and may eventually senesce (Gottwald *et al.* 2007). Therefore the bacteria would reproduce and survive for this period of time in infected plants.
- The survival and multiplication of this bacterium is affected by temperature. '*Candidatus* Liberibacter americanus' survives and produces symptoms at 22–24 °C

while higher temperatures 27–32 °C inhibits the infection (Gasparoto *et al* 2008; Lopes *at al*. 2009a).

- '*Candidatus* Liberibacter americanus' was not affected by a temperature of 24 °C when infected plants were maintained for 60 or 90 days. Multiplication of '*Ca.* L. americanus' is severely inhibited at 32 °C. Infected plants maintained at 27–32 °C after 60 days were negative for the presence of the bacterium by PCR and they remained bacterium free for two years (Lopes *et al*. 2009a). Infected plants maintained at 22–24 °C either senesced or had strong symptoms, indicating that 27–32 °C had reduced or eliminated the bacterium (Lopes *et al*. 2009a).
- In São Paulo State the characteristic blotchy mottle leaf symptoms are more prominent in the autumn and winter when minimum temperatures are cool and daily maximums rarely exceed 32 °C, and are thus favourable for '*Ca.* L. americanus' multiplication and symptom expression (Lopes *et al*. 2009a). It is well known that strong HLB fruit and leaf symptoms are associated with high *Ca.* L. species' titres (Teixeira *et al.* 2008).

- Outside a host plant, based on the persistent survival of '*Ca*. L. species' in *D. citri* (Bové 2006), '*Ca*. L. americanus' is likely to survive in the insect vector for a long time. The duration of the psyllid lifecycle from egg to adult is up to 47 days depending upon food supply and ambient temperature (Knapp *et al*. 2006); and adults of *D. citri* are known to survive 8–9 months over winter on suitable hosts (Yang *et al*. 2006). Therefore, '*Ca*. L. americanus' has the potential to survive within the psyllid vector for considerable periods of time.

***Cultural practices and control measures***

- There are currently no control measures in commercial orchards that target '*Ca* L. americanus' and no programs exist for garden, amenity or native hosts. Therefore current control measures are unlikely to impact on the establishment of '*Ca* L. americanus' in Australia.
- In Brazil, '*Ca*. L. americanus' is controlled by the elimination of all symptomatic trees to reduce inoculum sources and control of the insect vector (Lopes *et al*. 2007).
- Eradication may be possible if the bacterium is detected early and in the absence of psyllid vectors. However, there are no cases of successful eradication programs for '*Ca*. L. species' (Polek *et al.* 2007).
- Since the bacterium cannot be eliminated from affected tress, all trees showing symptoms are removed and destroyed (Floyd and Krass 2006). Treatment with antibiotics suppresses the bacterial symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

'*Candidatus* Liberibacter americanus' is able to survive and multiply within suitable hosts and its psyllid vectors, and survives in diverse climatic conditions supports an assessment of 'high' for the establishment of '*Ca.* L. americanus' in Australia.

### 5.2.3 Probability of spread

The likelihood that '*Candidatus* Liberibacter americanus' will spread based on a comparison of factors in the area of origin and in Australia that affect the expansion of the geographic distribution of the pest is **HIGH (VERY LOW[7])**.

---

[7] In the absence of suitable psyllid vectors

***The suitability of the natural or managed environment for natural spread***

- '*Candidatus* Liberibacter americanus' has a restricted distribution and is known only from Brazil (Teixeira *et al*. 2005b). There are similarities in the natural and urban environments of these areas with those in Australia, which suggests that '*Ca*. L. americanus' could spread in Australia (Peel *et al.* 2007).
- '*Candidatus* Liberibacter americanus' is known to be inactivated by temperatures above 32 °C (Lopes *et al*. 2009a). In Brazil, the climatic range of '*Ca* L. americanus' is limited to more temperate regions, and even though host plants and vectors are abundant throughout São Paulo State, the bacterium has not spread into the hotter regions of the state (Lopes *et al*. 2009a). This indicates that '*Ca*. L. americanus' is vulnerable to hot dry conditions (Lopes *et al*. 2009a) and is likely to be restricted in range to the cooler regions of Australia.
- '*Candidatus* Liberibacter species' were first reported in March 2004 in two municipalities and, as of August 2008, had spread to 241 municipalities of São Paulo, Minas Gerais and Paraná states (Lopes 2009) indicating that this pathogen is able to spread in natural or managed environments.
- Host plants that support the spread of '*Ca*. L. americanus' are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment. Hosts include all species and cultivars of citrus and some non-citrus Rutaceae (da Graça 2008; Lopes and Frare 2008).
- Natural spread of '*Ca.* L. species' is dependent on the transmission of the bacterium by its psyllid vectors (Rogers *et al.* 2008). In the absence of vectors, natural spread is limited or absent (Polek *et al.* 2007).
- In the absence of vectors, spread of '*Ca.* L. species' would rely on the movement of infected propagative material (Polek *et al.* 2007). However, the bacterium is unable to spread from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Presence of natural barriers***

- Hosts of '*Ca.* L. americanus' are present in many parts of Australia (APNI 2008). Natural barriers, such as arid areas, mountain ranges, climatic differentials and possible long distances exist between suitable hosts and may prevent long-range natural spread of this bacterium.
- The Australian citrus industry is spread across Australia in isolated regions with long distances often separating commercial orchards. This will aid in containment and eradication of the vector and/or pathogen if establishment occurs in an isolated citrus growing region (Barkley and Beattie 2008).
- '*Candidatus* Liberibacter americanus' is heat sensitive (Lopes *et al*. 2009a) and arid regions surrounding many *Citrus* species production areas in Australia would prove to be a natural barrier to the spread of this bacterium.
- The bacterium has an uneven distribution in São Paulo State. From its initial detection, '*Ca*. L. americanus' has moved longer distances to the south than to the north/northwest, despite the widespread presence of its vector and many citrus orchards (Lopes *et al.* 2007). The south is characterized by lower air temperatures than the north/northwest. The heat sensitivity of '*Ca.* L. americanus' explains the absence of the bacterium in the hot northwest region of São Paulo State (Lopes *et al*. 2009b). This suggests that the bacterium would have trouble spreading in the more arid to tropical regions of the Australia.

- A significant natural barrier to spread is the absence of suitable vectors of the bacterium in the PRA area. Natural spread of '*Ca*. L. americanus' is achieved by *D. citri* (Teixeira *et al.* 2005b), which is not present in Australia.

***Potential for movement with commodities or conveyances***

- '*Candidatus* Liberibacter americanus' is spread to new areas by the introduction of infected plant material (Bové 2006). As visual symptoms may not be present, and in the absence of specific testing regimes, infected nursery stock could easily be moved to new areas.
- The introduction of infected plant material establishes the pathogen in new areas and, if unregulated movement occurs, it will accelerate the spread of the pathogen (Gottwald *et al*. 2007).
- Graft transmission has been demonstrated for '*Ca*. L. americanus' but this bacterium does not transmit efficiently by grafting (Lopes *et al*. 2009b). Graft transmission is variable depending on the plant part used for grafting and the amount of tissue used (Van Vuuren 1993; Halbert and Manjunath 2004).
- The most appropriate source of inoculum to be used for transmission was 4 cm long bud sticks removed from symptomatic branches. Transmission efficiencies ranged from 25–65.2%. Single buds and 2 cm long bud sticks were less effective, and transmission efficiency ranged from 10–17.1% (Lopes and Frare 2008).
- Transmission efficiencies of peduncle bud sticks ranged from 15–37.5%. However, tissue survival is an important factor in graft transmission. Tissue survival rate is lower in cooler winter months; however, pathogen transmission efficiency is higher during the winter (Lopes and Frare 2008).
- '*Candidatus* Liberibacter americanus' was detected on scions developed from buds of symptomatic branches, but not from asymptomatic branches of the same affected trees. This indicates that the chances of disseminating '*Ca.* L. americanus' via nursery stock propagated from asymptomatic trees is low (Lopes *et al*. 2009b).
- There is also potential for the spread of the bacterium through infected seed. However, research has demonstrated that while a proportion of seedlings that develop from seed from infected plants are positive for the bacterium and a proportion of these positive seedlings develop symptoms of citrus greening (Benyon *et al*. 2008; Graham *et al*. 2008; Hartung *et al*. 2008, Shatters 2008), the infection rate can decrease over time (Graham *et al*. 2008).
- Infected psyllid vectors could spread the bacterium through contaminating commodities and conveyances. *Diaphorina citri* can spread this bacterium through transport of infested plant material (Bové 2006; Gottwald *et al.* 2007).
- There are also reports from Brazil of *D. citri* being carried in empty fruit trucks (Beattie and Barkley 2009) indicating that an infected psyllid has the potential to move with commodities or conveyances.

***Potential natural enemies***

- '*Candidatus* Liberibacter species' are not known to have any natural enemies that could hamper their spread. However, certain entomophagous insects can drastically reduce psyllid populations and thus indirectly prevent an increase of the pathogen population and associated spread (Aubert 1987a).

**Spread potential if known vectors establish in Australia**[8]

- *Diaphorina citri* is the natural vector of '*Ca.* L. americanus' (Teixeira *et al.* 2005b) and acquires the bacterium while feeding on infected plants (Lopes and Frare 2008). Once the psyllid has acquired the bacterium from infected hosts, based on the persistent survival of related '*Ca*. L. species' in *D. citri* and *T. erytreae* (Bové 2006), the vector is likely to maintain the ability to spread the bacterium throughout its life.
- Spread of the bacterium in the presence of the vector can be dramatic. For example, in a two year old orchard surrounded by heavily infected adult trees, the disease incidence increased from 0.6% to 27.4% in 9–10 months (Gottwald *et al*. 2007). Disease progression can also be quite fast, reaching more than 95% incidence in 3–13 years after the first symptoms occur (Gottwald *et al*. 2007). Therefore, in the presence of inoculum and the vector, the bacterium would be spread quickly in more southern regions of Australia (Barkley and Beattie 2008).
- '*Candidatus* Liberibacter species' were first reported in March 2004 in two municipalities in São Paulo State and, as of August 2008, had been spread to 182 municipalities (Lopes *et al*. 2009a) and, as of March 2009, had spread to 241 municipalities of São Paulo, Minas Gerais and Paraná states of Brazil (Lopes 2009).
- In the presence of *D. citri* the rate of spread of '*Ca.* L. species' has been estimated to be 12 miles (19 km) per year (Gottwald *et al.* 2007).
- The natural spread of '*Ca*. L. americanus' by psyllid vectors in endemic areas will depend on acquisition period, latency of the pathogen in the psyllid prior to transmission and transmission efficiency (Rogers *et al*. 2008).
- '*Candidatus* Liberibacter americanus' does not multiply efficiently in plants and therefore its titres are low (Lopes *et al.* 2009b). Lower titres of this bacterium in the host plants decrease the likelihood of acquisition and transmission by the insect vector (Lopes *et al*. 2009a).
- *Diaphorina citri* is an efficient vector of '*Ca*. L. americanus' (Teixeira *et al.* 2005b). However, there is little information in the literature regarding the components of the vector-pathogen interaction. *Diaphorina citri* can acquire '*Ca.* L. asiaticus' after 30 minutes of feeding with a latent period of 1–21 days (Halbert and Manjunath 2004).
- Fourth and fifth instar nymphs of *D. citri* can acquire '*Ca*. L. species' and are able to transmit them immediately, once they mature to adults (Halbert and Manjunath 2004).
- '*Candidatus* Liberibacter americanus' is heat sensitive (Lopes *et al*. 2009a) whereas *D. citri* is heat tolerant (Bové 2006). For example '*Ca*. L. americanus' spreads in the cooler south region, whereas it is absent in the hotter north/northwest region of São Paulo State, Brazil (Lopes *et al*. 2009a).
- Cooler areas, including sub-tropical climates of high lying areas, would be suitable for the spread of the pathogen, while the psyllid is better adapted to regions with high temperatures and low relative humidity (Halbert and Manjunath 2004). This suggests that the bacterium would have trouble spreading in the more arid to tropical regions of Australia, even in the presence of the psyllid vector. Therefore the spread of the pathogen by the psyllid would be more likely in the temperate southern regions of Australia and not in the warmer tropical northern regions (Barkley and Beattie 2008).
- The presence of natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances between suitable hosts, may limit the ability of *D. citri* to spread '*Ca*. L. americanus' to new areas.

[8] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca.* L. americanus' in the presence of known vectors.

- *Diaphorina citri* is capable of moving independently at least 8–60 m (Hall 2008a) and adults have been estimated to be transported in wind drifts for 0.5–1 km (Barkley and Beattie 2008). Furthermore, there is some evidence for occasional adult mass migrations (Hall 2008a).
- Bass Strait may act as a natural barrier to *D. citri* carrying '*Ca.* L. americanus' from mainland Australia to Tasmania as the psyllids would not be capable of dispersing the distance over the strait. However, *D. citri* also have the potential to move large distances (over 90–470 km) on seasonal trade winds or hurricanes (Bové 2006; Sakamaki 2005; Tsai 2006; Gottwald *et al*. 2007; Halbert *et al*. 2008).
  - Field studies in the Philippines and China suggest possible medium to long distance transport of *D. citri* by strong winds in open orchards without windbreak protection (Aubert 1987a).
  - It is speculated that migration of the psyllid in the Okinawan Islands in southeast Japan is governed by seasonal winds. It possible for the psyllid to migrate 470 km northwards to the large island of Kyushu over sea by riding lower jet airstreams associated with summer monsoons (Sakamaki 2005).
  - It is also speculated that *D. citri* may have been carried some 90–145 km by wind over non-citrus growing regions in Florida. This long-distance movement may have been related to air masses during hurricanes or tropical storms (Gottwald *et al*. 2007).
  - There is circumstantial evidence that infected *D. citri* flew across the Florida Everglades and infected the eastern borders of large commercial citrus groves just to the west of the Everglades (Manjunath *et al*. 2008).
- Passive movement of the psyllid indicates that infected psyllids could overcome the natural barriers in Australia and spread the bacterium in suitable environments.
- *Diaphorina citri* feeds and reproduces on the Rutaceae, including cultivated *Citrus* species and Australian naturalised/native species *Murraya paniculata* and *Citrus australasica* (Halbert and Manjunath 2004). These hosts are widespread in commercial, natural and urban environments of the PRA area. Most, but not all, host species of *D. citri* are also hosts of '*Ca*. L. americanus'. This makes it increasingly likely that the vector would spread '*Ca*. L. americanus' to suitable hosts.
- Suitable hosts of *D. citri* and the bacterium are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- Once '*Ca.* L. species' are detected in a citrus orchard, in the absence of control measures, known vectors have been recorded to spread the pathogen to 100% of trees in five years (Bové 2006).

The suitability of the environment in temperate Australia, presence of multiple host species throughout the PRA area, potential to disperse in propagative material and potential for spread with the known vector supports an assessment of 'high' for the spread of '*Ca.* L. americanus'.

**Spread potential in the absence of known vector[9]**

- In the absence of known vectors, the most likely means of spread of '*Ca.* L. americanus' would be through the movement of infected planting material. If infected budwood is used for grafting it will help spread the bacterium to non-infested areas.

[9] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca.* L. americanus' in the absence of known vectors.

- If '*Ca*. L. americanus' was to establish in Australia, in the absence of vectors, it would likely lead to the death of its infected host plants and not pose a severe threat to the citrus industry (Barkley and Beattie 2008).
- Although there is one species of *Diaphorina* (*D. tryoni*) present in Australia, this species has not been recorded on the Rutaceae (DEWHA 2009). It is unlikely that this species would act as a vector for the disease.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on citrus, where '*Ca*. L. species' are present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to act as vectors.

In the absence of *D. citri* from Australia, the assessment supports a rating of 'very low' for the spread of '*Ca* L. americanus'.

### 5.2.4 Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probability of entry, of establishment and of spread using the matrix of rules for combining descriptive likelihood (Table 2.2).

- The overall likelihood that '*Ca.* L. americanus' will enter Australia by the pathways discussed in this PRA, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia are set out in Table 5.5.

**Table 5.5: Overall probability of entry, establishment and spread of '*Ca.* L. americanus' by different pathways**

| Pathway | Probability of | | | Overall probability of entry, establishment and spread |
|---|---|---|---|---|
| | Entry | Establishment | Spread | |
| Fruit (containing seed) | Extremely low | High | High (Very low)* | Extremely low (Extremely low) |
| Nursery stock (live plants) | High | | | High (Very low) |
| Budwood | Moderate | | | Moderate (Very low) |
| Fresh leaves | Extremely low | | | Extremely low (Extremely low) |
| Seed for sowing | Low | | | Low (Very low) |
| Infected psyllids | Moderate | | | Moderate |

* Ratings in parenthesis are in the absence of known vectors in Australia

### 5.2.5 Consequences

The consequences of the entry, establishment and spread of '*Ca.* L. americanus' in Australia have been estimated according to the methods described in Table 2.3. The assessment of potential consequences for the bacterium is treated in combination with its psyllid vector as HLB has never been detected in the absence of a vector and economic consequences have never been assessed in isolation. The assessment is provided below:

| Criterion | Estimate and rationale |
|---|---|
| **Direct** | |
| Plant life or health | **Impact score**: **E** – Significant at the regional level.<br>• '*Ca*. L. species' are highly destructive pathogens and are major limiting factor for citrus production. The impact of '*Ca.* L. americanus' is increasing in the Americas as the pathogen and its vector spread through the region.<br>• The presence of *Ca.* L. americanus' in Brazil has led to the elimination of an estimated three million diseased trees since '*Ca*. L. americanus' and '*Ca*. L. asiaticus' were described from São Paulo State, in 2004 (Lopes *et al*. 2008).<br>• The introduction of *Ca*. L. species' and its vector into a climatically suitable area could result in the destruction of the citrus industry of that region, while in areas where the disease was endemic, citrus trees could live for 5–8 years (Roistacher 1996).<br>• The bacterium infects both plants and fruit (Aubert 1987a). Trees infected at an early age may fail to produce fruit while more mature trees become unproductive soon after infection.<br>• Infected fruit are small, lopsided, remain green at the stylar end, contain aborted seed and discoloured vascular bundles, and have a bitter taste, probably because of higher acidity and lower sugars (da Graça 1991). Many fall prematurely (da Graça 1991) and may taint extracted juice products with consequent economic loss.<br>• Some Australian Rutaceae has been tested for susceptibility to '*Ca*. L. species' (Halbert and Manjunath 2004). Several native and naturalised species demonstrate disease symptoms following natural infection and many more species are suspected as being hosts but have not undergone susceptibility trials.<br>• None of the native Australian Rutaceae considered potential hosts of '*Ca.* L. species' (Beattie and Barkley 2009) are rare or endangered (DEWHA 2009).<br>• Some hosts of '*Ca*. L. americanus' constitute a component of garden environments. Such hosts are sporadically distributed however; any impact would be restricted to the local levels. |
| Other aspects of the environment | **Impact score**: **B** – Minor significance at the local level.<br>There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>• In general, newly established species may affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants, degrade critical habitat or stimulate use of chemicals or biological controls. There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants. '<br>  – '*Candidatus* Liberibacter americanus' is unlikely to affect the environment in these ways, as the bacterium infects and multiply only in the Rutaceae. |
| **Indirect** | |

| Criterion | Estimate and rationale |
|---|---|
| Eradication, control etc. | **Impact score**: **F** – Significant at the national level.<br>• Huanglongbing symptoms abate temporarily when trees are injected with antibiotics (Halbert and Manjunath 2004). Consequently prevention is the only reliable control method.<br>• Once established, '*Ca*. L. americanus' is both difficult and expensive to eradicate. Eradication of the bacterium would require the removal of large numbers of native, amenity and commercial citrus plants within the vicinity of outbreaks. Due to the large number of host plants affected, the costs of any eradication campaign are likely to be substantial.<br>• General control methods for '*Ca*. L. americanus' include certified pathogen-free nursery trees, systemic insecticides to control the psyllid vector, *D. citri*, and removal of diseased trees to reduce inoculum levels (Lopes *et al.* 2008). Implementing these measures would require a multifaceted and costly approach.<br>• While potentially able to be managed in commercial production, the presence of the bacterium will significantly increase the production costs for producers. For example, in Florida it is estimated that production costs associated with an aggressive management program for *D. citri* and '*Ca*. L. asiaticus' are 40% greater than the pre-HLB costs. Although '*Ca*. L. americanus' is considered to be less aggressive than '*Ca*. L. asiaticus', increased production costs could approach these levels.<br>• Control of the psyllid vector is the key to limiting the impact of pathogen. Foliar sprays (imidacloprid) have been used to control the psyllid vectors of the disease (Grafton-Cardwell *et al*. 2006).<br>• Considering the extreme fertility of the psyllid vector with each female laying as many as 1000 to 2000 eggs in a matter of three weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment (Aubert 2008). |
| Domestic trade | **Impact score**: **D** – Significant at district level<br>• The presence of '*Ca.* L. americanus' in production areas may result in some domestic movement restriction for host commodities. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock and seed for sowing.<br>• Interstate restrictions on nursery stocks of the Rutaceae may lead to a loss of markets, which in turn would be likely to require industry adjustment. |
| International trade | **Impact score**: **D** – Significant at district level<br>• The presence of '*Ca.* L. americanus' in production areas may limit access to some overseas markets where the pathogen is absent. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock and seed for sowing.<br>• Brazil, where this bacterium occurs, still exports fruit to several destinations. |
| Environmental and non-commercial | **Impact score**: **C** – Significant at the local level.<br>• Large scale removal of infected native and amenity host plants would have significant effects on the environment. Broad-scale chemical treatments directed against known insect vectors may also have some impacts on native insects.<br>• Historically, the introduction of invasive agricultural pests has initiated control measures to avoid lost production. Consumer preferences for unblemished, high quality produce encourages the use of pesticides, while at the same time, negative public opinion regarding the use of pesticides on fruits and vegetables is a market concern (Bunn *et al*. 1990). Therefore, the establishment of any new pests of fruits and vegetables destined for fresh markets is likely to stimulate greater use of either chemical or biological controls to ensure market access.<br>• Twenty insecticide applications per year would be required to control the psyllid vector (Lopes *et al.* 2008). Wind dispersal of dry orchard soil containing broad spectrum chemicals may also have other impacts on the local environment. |

Based on the decision rules described in Table 2.4, where the consequences of a pest with respect to one or more criteria are '**F**', the overall consequences are considered to be **HIGH**.

### 5.2.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for '*Ca*. L. americanus' is summarised in Table 5.6.

**Table 5.6: Unrestricted risk estimates of '*Ca*. L. americanus' for different pathways**

| Pathway | Overall probability of entry, establishment and spread | Consequences | Unrestricted risk |
|---|---|---|---|
| Fruit (containing seed) | Extremely low (Extremely low) | High | Very low (Very low) |
| Nursery stock (live plants) | High (Very low) | | High (Low) |
| Budwood | Moderate (Very low) | | High (Low) |
| Fresh leaves | Extremely low (Extremely low) | | Very low (Very low) |
| Seed for sowing | Low (Very low) | | Moderate (Low) |
| Infected psyllids | Moderate | | High |

* Ratings in parenthesis are in the absence of known vectors in Australia

The unrestricted risk for '*Ca*. L. americanus' has been assessed as 'high–low' for nursery stock, budwood, 'moderate–low' for seed for sowing and 'high' for psyllid vectors, which are all above Australia's ALOP. Therefore, specific risk management measures are required for '*Ca*. L. americanus' for nursery stock, budwood, seed for sowing and psyllid vectors. The unrestricted risk for '*Ca*. L. americanus' has been assessed as 'very low' for fruit and fresh leaves, which is below Australia's ALOP. Therefore, no management measures for these pathways are required.

## 5.3 '*Candidatus* Liberibacter asiaticus'

Symptoms consistent with HLB in citrus were reported in the mid 1700s in the central Indian provinces and presence of the disease in north-western and north-eastern India was reported in the 1800s and 1900s (Beattie *et al*. 2008). These records suggest that '*Candidatus* Liberibacter asiaticus' may have originated in India (Halbert and Manjunath 2004) and evolved there in association with *Murraya paniculata*, *Murraya koenigii* or species of *Clausena*, but not species of *Citrus*, as no true species of *Citrus* are native to India (Bayer *et al*. 2009). Natural infection by '*Ca*. L. asiaticus' has only been detected in the Aurantioideae subfamily (Beattie *et al*. 2008).

'*Candidatus* Liberibacter asiaticus' is heat tolerant and occurs under low humidity and at both cool and warm temperatures (Bové 2006). The natural vector of this pathogen, *Diaphorina citri* (Capoor *et al*. 1974), shares the temperature preference of the bacterium. *Diaphorina citri* thrives in warm environments and is also tolerant of high temperatures (Bové 2006). Geographical variation in '*Ca*. L. asiaticus' has been reported (Garnier *et al*. 1991; Teixeira *et al*. 2005b; Doddapaneni *et al*. 2008; Zhou *et al*. 2008a; Tomimura *et al*. 2009) and severe symptoms have been observed in hosts, including those hosts that until recently had been considered tolerant, e.g. pomelos (Tsai *et al*. 2008).

### 5.3.1 Probability of entry

### Pathway 1—Fruit (containing seed)

Seedless fruit poses equal or less risk than fruit containing seed. Therefore, only fruit containing seed has been considered in the pathway analysis for '*Candidatus* Liberibacter species' as the analysis is also relevant to seedless fruit.

#### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia with the trade in fresh fruit (containing seeds) for consumption from countries where the pathogen is present is **VERY LOW**.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter asiaticus' is associated with cultivated and ornamental Rutaceous host plants and infects both fruits as well as trees (Bové 2006; Gottwald *et al.* 2007; da Graça 2008) and is therefore associated with the pathway.
- Trees that become infected with '*Ca*. L. species' in the nursery or before reaching fruit bearing age tend to have new growth that becomes systemically infected and the trees fail to produce fruit (Pretorius and Van Vuuren 2006; Gottwald *et al*. 2007).
- Fruit sourced from symptomatic plants may contain the bacterium. Infected fruits are notably symptomatic (da Graça 1991). Fruits are small and lopsided, exhibit strong colour inversion, seed abortion and have brown/orange stained vascular bundles (Bové 2006). There is excessive fruit drop in HLB infected trees, while fruits that remain on the tree, especially sweet oranges, can have a mottled appearance (Gottwald *et al*. 2007).
- Consumer demand for high quality citrus fruit, including the appearance and shape of the fruit, have been important in raising citrus fruit standards internationally (UNCTAD 2006). The grading of citrus fruit to meet quality standards has been defined by the international standard setting body, Codex Alimentarius. To meet internationally agreed quality standards (Codex 2005), symptomatic fruit from commercial orchards are likely to be removed during grading operations.
- Based on PCR testing of fruit parts, the peduncle, columella and seed coat from infected trees were found to contain the bacterium (Tatineni *et al*. 2008). However, the bacterium was not detected in the endosperm and embryo of seeds from infected plants (Tatineni *et al*. 2008).
- The bacterium can also be retained in floral parts (petals, pistils and stamens) and could advance to young fruit during fruit development (Tatineni *et al*. 2008). For example, asymptomatic fruits sourced from symptomatic branches were considered to be in the early stages of colonization as the bacterium was only detected in the peduncle and central axis (Li *et al*. 2009). The bacterium was not detected in the pericarp or mesocarp, endocarp or locular membranes of the pre-symptomatic fruits (Li *et* al. 2009).
- The peduncle is known to carry a high concentration of the bacterium (Tatineni *et al*. 2008). High levels of peduncle infection are likely to be associated with symptomatic fruit.
- Generally, the bacterium is detected only in symptomatic fruits. Fruit is known to harbour lower concentrations of the bacterium (Floyd and Krass 2006). For example, '*Ca.* L. asiaticus' was detected in the fruits but the population levels were 1000-fold less than the populations in the midribs, leaf blades, bark and roots of infected trees (Li *et al*. 2009b).

- The detection of '*Ca* L. asiaticus' in fruit harvested from asymptomatic branches has not been recorded.
- The distribution of '*Ca.* L. asiaticus' within the host tissues is variable (Tatineni *et al*. 2008), with the level of infection in symptomatic fruit being comparatively low (Li *et al*. 2009). Based on this knowledge, the level and incidence of bacterial infection of asymptomatic fruit, particularly from healthy trees, is likely to be low.

***Ability of the pest to survive transport and storage***

- Fruit destined for Australia would be shipped in refrigerated containers maintained at 4–6 ºC; conditions that are unlikely to adversely affect '*Ca*. L. asiaticus' that are present during shipment.
- '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008). For example, the bacterium was detected in plant samples stored at 4 ºC for two months (Li *et al*. 2008). Although the viability of the bacteria was not confirmed, Li *et al*. (2008) demonstrated that over a 10 week period of testing the quantity of bacterium in plant samples did not change, which may indicate the presence of some viable bacteria.
- Based on this information it is concluded that cold storage conditions are likely to stop bacterial multiplication, but are unlikely to have any impact on the survival of '*Ca.* L. asiaticus' in imported fruit.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated (Floyd and Krass 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The abortion of most infected fruits, obvious symptoms of infection, international quality standards that exclude fruit damaged by pests and no known history of interception in fruit supports an assessment of 'very low'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed in a viable state within Australia with imported fruit (containing seed) from countries where the pathogen is present and transferred to a suitable host is **EXTREMELY LOW**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter species' are unable to move independently to a suitable host. Movement of '*Ca.* L. asiaticus' to a suitable host is only possible by grafting or by psyllid vectors (Gottwald *et al*. 2007). Fruit and fruit parts are not suitable for grafting.
- Without grafting, the movement of '*Ca.* L. asiaticus' from imported fruit to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. asiaticus' is vectored by *Diaphorina citri* and *Trioza erytreae* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.

- Although five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, none of these species have been recorded on the Rutaceae (DEWHA 2009).
- Furthermore, even if psyllid vectors are present in Australia, they preferentially feed on new flushes of growth (Halbert and Manjunath 2004) and may not feed on imported fruits. Currently there is no information to support the ability of psyllid vectors to transmit '*Ca.* L. asiaticus' from infected host fruit to healthy host plants.
- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Halbert and Manjunath 2004; Bové 2006; Lopes and Frare 2008). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.

***Distribution of the imported commodity in the PRA area***

- Imported fruits will be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the fruit is likely to reach areas of host abundance. Distribution of infected fruit would facilitate the distribution of '*Ca.* L. asiaticus'.
- '*Candidatus* Liberibacter asiaticus' would need to survive transportation and storage within the PRA area. '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008). Thus, transport and storage conditions are unlikely to have any impact on the level of '*Ca.* L. asiaticus' infection in fruit distributed for retail or market sale.

***Risks from by-products and waste***

- Although the intended use of fresh fruit is human consumption, waste material would be generated (e.g. overripe and damaged fruit, uneaten portions). Whole or parts of the fruit may be disposed of at multiple locations throughout Australia in compost bins or amongst general household or retail waste.
- '*Candidatus* Liberibacter asiaticus' has been detected in infected fruit parts including the peduncle, columella, seed coat and fruit peel (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem-limited bacteria.
- Although '*Ca*. L. asiaticus' is considered heat-tolerant (Bové 2006; Lopes *et al*. 2009b), composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. asiaticus' is heat-sensitive to temperatures above 38 °C (Lopes *et al*. 2009a).
- A relatively high proportion of household and retail waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. asiaticus' from the household and environment, reducing the likelihood that susceptible plants will be exposed to this bacterium.
- If psyllid vectors are present in Australia, they preferentially feed on new flush and may not feed on fruit waste.
- Seeds present in fruit waste will desiccate quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, seed may not germinate because they do not tolerate moisture loss.
- If a plant was to establish from seed disposed of in a compost bin or amongst general household waste, the seedlings would then be subject to ambient conditions, including variable moisture, temperature extremes (heat and frost) and plant competition, which

will reduce seedling survival. Furthermore, seed to seedling transmission would be critical for the bacterium to enter the environment.

- Seed to seedling transmission has been reported based on symptoms (Tirtawidjaja 1981). However, as '*Ca.* L. asiaticus' is found only in the seed coat and not in the seed endosperm and embryo, it has been questioned how the bacterium enters the phloem of seedlings (Tatineni *et al*. 2008).
- Recent research suggests that while a proportion of seedlings that develop from seed from infected plants are positive for the bacterium, the infection is transient and may be lost over time (Graham *et al*. 2008; Shatters 2008).
- In the absence of a suitable vector the bacterium is unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

The desiccation of citrus seed, the variable and likely unsuitable conditions for seedlings, transient seed transmission and inability of '*Ca.* L. asiaticus' to be distributed in the absence of a vector or graft transmission supports an assessment of 'extremely low'.

## Pathway 2—Nursery stock (live plants)

### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia with trade in nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter asiaticus' has been reported in association with cultivated and ornamental Rutaceous host plants and can be associated with all vegetative parts of host plants (Tatineni *et al.* 2008; Lopes *et al*. 2009a). Therefore nursery stock of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter asiaticus' can have a long latent period and many infected plants remain symptomless, particularly those that are newly infected (Xu *et al*. 1988b; Wang *et al*. 1996; Gottwald *et al*. 2007). It is highly likely that nursery stock from countries where this bacterium is present will contain the bacterium.
- '*Candidatus* Liberibacter asiaticus' survives within the living phloem cells, in the vascular system of host plants (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant that other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).
- As the bacterium infects phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). '*Candidatus* Liberibacter asiaticus' is an aggressive pathogen and is able to produce severe symptoms under favourable conditions (da Graça 1991). Symptom expression is influenced by temperature and varies with season, and in unfavourable environments symptoms may not be expressed (Bové 2006).
- Initial symptoms caused by '*Ca*. L. asiaticus' are difficult to distinguish from nutrient deficiencies or other plant diseases (Li *et al*. 2009). Typical foliage symptoms include pale yellowing and blotchy mottle (Kim *et al*. 2009). Infected leaves can become upright, followed by leaf drop at the laminar abscission zone and twig dieback at a later stage (Kim *et al*. 2009).
- '*Candidatus* Liberibacter species' can be detected in asymptomatic host foliage only when the bacterial titre is above a certain level (Li *et al.* 2008; Manjunath *et al.* 2008). However, '*Ca.* L. species' are difficult to detect because they are often present at a low

concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).

- Infected nursery stock may be asymptomatic in the initial stages of infection and can be easily overlooked. It is reported that 15–20% of infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- '*Candidatus* Liberibacter asiaticus' is able to multiply efficiently in citrus plants (Lopes *et al*. 2009a) and its titres in hosts are high (Lopes and *et al*. 2009b).
- Association with nursery stock can provide long term survival for '*Ca*. L. species'. If young trees are infected, the bacterium has ample time to colonise different parts of the plant (Li *et al*. 2009). It is likely that asymptomatic plants infected by '*Ca.* L. asiaticus' would pass routine visual inspections and be released into Australia.

***Ability of the pest to survive transport and storage***

- Nursery stock is expected to be shipped at moderate temperatures and humidity levels to ensure nursery stock survival; conditions which are unlikely to adversely affect any '*Ca*. L. asiaticus' that are present during shipment.
- Long-term storage of plant samples at 4 ºC for two months does not affect the detection of '*Ca*. L. asiaticus' bacteria (Li *et al*. 2008). Although the viability of the bacteria was not confirmed, Li *et al*. (2008) demonstrated that over a 10 week period of testing the quantity of bacterium in plant samples did not change, which may indicate the presence of some viable bacteria.
- '*Candidatus* Liberibacter asiaticus' has been introduced into new areas by the importation of infected planting material (Bové 2006).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated from a plant (Floyd and Krass 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The association of '*Ca*. L. asiaticus' with nursery stock, the ability for infected plants to remain asymptomatic and the likelihood of this bacterium remaining viable during transport and storage support an assessment of 'high'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed within Australia with imported nursery stock (live plants) from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter asiaticus' arriving in Australia with nursery stock will not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host.
- Natural movement of '*Ca*. L. species' from infected nursery stock to a suitable host, in the absence of vectors, is limited or absent (Polek *et al.* 2007). The bacterium can move to a suitable host only by grafting (Aubert 2008).
- Without grafting, the transfer of '*Ca.* L. asiaticus' from imported nursery stock to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector

specific and '*Ca.* L. asiaticus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.

- Nursery stock is imported specifically for the purpose of propagation. Infected nursery stock is therefore likely to be planted directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported nursery stock.
- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Halbert and Manjunath 2004; Bové 2006). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to these hosts in the PRA area.
- Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, but none of these species have been recorded on the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- In the absence of vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- The distribution of nursery stock would be for retail distribution to multiple destinations throughout Australia. Nursery stock would also be distributed for breeding and propagation. Distribution of infected nursery stock commercially through nurseries would facilitate the distribution of '*Ca*. L. asiaticus'.
- Infected nursery stock may be distributed to glasshouses or retail shops where the bacterium may continue multiplying within the host. Infected nursery stock is unlikely to be grown in isolation, providing greater opportunity for the bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected nursery stock to other hosts.
- '*Candidatus* Liberibacter asiaticus' would need to survive transportation and storage within the PRA area. Nursery stock is expected to be maintained at moderate temperatures and humidity levels to ensure nursery stock survival, so a portion of infected propagation material that enters the country is likely to reach areas of host abundance.
- As nursery stock may not display obvious symptoms of '*Ca.* L. asiaticus' infection, there is a risk that infected plant material would be used for propagation. Material from infected plants may be used for grafting and grafted rootstock is planted directly at multiple locations in Australia.
- Asymptomatic plants may also be overlooked and sold to commercial users and households.

***Risks from by-products and waste***

- Although the intended use of nursery stock is for propagation, and all imported material would be grown under ideal conditions, waste material may be generated. Whole or parts of the plants may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.

- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem-limited bacteria.
- Although '*Ca*. L. asiaticus' is considered heat-tolerant (Bové 2006; Lopes *et al*. 2009b), composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. asiaticus' is heat-sensitive to temperatures above 38 °C (Lopes *et al*. 2009a).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste material. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However, acquisition and transmission times for *D. citri* vary from 1–25 days (Halbert and Manjunath 2004).

The association of '*Ca.* L. asiaticus' with asymptomatic nursery stock and the likely distribution of infected plants to multiple locations supports an assessment of 'high' for the distribution of this species in nursery stock.

## Pathway 3—Budwood

### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia with trade in budwood from countries where the pathogen is present is **MODERATE**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter asiaticus' has been reported in association with cultivated and ornamental Rutaceous host plants and can be associated with all vegetative parts of host plants (Tatineni *et al.* 2008; Lopes *et al*. 2009a). Therefore budwood can provide a pathway for the importation of '*Ca.* L. asiaticus' into Australia.
- '*Candidatus* Liberibacter asiaticus' survives within the living phloem cells, in the vascular system of host plants (Kim *et al*. 2009). The location of the bacterium in the phloem allows it to avoid the natural defences of the plant, which other pathogens encounter when they infect foliar and intercellular spaces (Kim *et al*. 2009).
- Since '*Ca*. L. species' infect phloem, once a plant has become infected the bacterium can move throughout the plant (Spann *et al*. 2008). However, the infection has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present all stems of infected trees.
- '*Candidatus* Liberibacter asiaticus' is an aggressive pathogen and is able to produce severe symptoms under favourable conditions (da Graça 1991). Typical foliage symptoms include pale yellowing and blotchy mottle (Kim *et al*. 2009). Infected leaves can become upright, followed by leaf drop at the laminar abscission zone and twig dieback at a later stage (Kim *et al*. 2009).
- Infected field trees can be identified by their foliar and fruit symptoms (Polek *et al*. 2007). Budwood is high value and a large investment is made in the health and survival of mother plants. Therefore, it is unlikely that budwood would be sourced from symptomatic trees.

- However, initial symptoms caused by '*Ca*. L. asiaticus' are difficult to distinguish from nutrient deficiencies or other plant diseases (Li *et al*. 2009). Furthermore, '*Ca.* L. asiaticus' can have a long latent period and many infected plants remain symptomless, particularly those which are newly infected (Xu *et al*. 1988b; Wang *et al*. 1996; Gottwald *et al*. 2007). It is therefore likely that budwood sourced from asymptomatic trees could contain low concentrations of the bacterium (Huang *et al*. 1999; Li *et al*. 2009).
- '*Candidatus* Liberibacter species' can be detected in asymptomatic hosts only when the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008). However, '*Ca.* L. species' are difficult to detect because they are often present at a low concentration and are unevenly distributed within their host tissues (da Graça 1991; Hung *et al*. 1999; Li *et al*. 2009).
- Infected mother plants may be asymptomatic in the initial stages of infection and can be easily overlooked when sourcing budwood. For example, it is reported that 15–20% of infected plants are overlooked by nursery inspectors who rely on visual inspection only (Halbert and Manjunath 2004).
- Once budwood has been harvested from infected trees the bacterium does not produce visible symptoms on budwood and may escape detection during routine visual inspection. Association with budwood can provide long term survival for the bacterium.

***Ability of the pest to survive transport and storage***

- Budwood is expected to be shipped at moderate temperatures and humidity levels to ensure survival; conditions which are unlikely to adversely affect any '*Ca*. L. asiaticus' present during shipment.
- Long-term storage of plant samples at 4 ºC for two months does not affect the detection of '*Ca*. L. asiaticus' bacteria (Li *et al*. 2008). Although the viability of the bacteria was not confirmed, Li *et al*. (2008) demonstrated that over a 10 week period of testing the quantity of bacterium in plant samples did not change, which may indicate the presence of some viable bacteria.
- '*Candidatus* Liberibacter asiaticus' has been introduced into new areas by the importation of infected planting material (Bové 2006).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated from a plant (Floyd and Krass 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

Although '*Candidatus* Liberibacter asiaticus' is associated with budwood and the bacterium would remain viable during transport and storage, the bacterium is unevenly distributed within host trees and may not be present in budwood sourced from infected asymptomatic hosts. Therefore, an assessment of 'moderate' for the importation of this species in budwood is allocated.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed within Australia with imported budwood from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter asiaticus' arriving in Australia with imported budwood will not need to move from the import pathway to a suitable host as the bacterium is already within a suitable host.
- '*Candidatus* Liberibacter asiaticus' is unable to move independently from imported budwood to a suitable host. Movement of '*Ca*. L. asiaticus' to a suitable host is only possible either by grafting or by psyllid vectors (Aubert 2008; Lopes *et al*. 2009b).
- Budwood is imported specifically for the purpose of grafting onto local rootstock, which will then be planted directly into a suitable habitat at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the grafted rootstock. Therefore the bacterium will move from imported budwood to a suitable host by grafting.
- Graft transmission has been demonstrated for '*Ca*. L. asiaticus' (Halbert and Manjunath 2004; Bové 2006). However, graft transmission rates are variable depending on the plant part used for grafting and the amount of tissue used (Halbert and Manjunath 2004). Therefore, the grafting technique and low bacterial titres will effect symptom expression and could prevent early detection.
- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Halbert and Manjunath 2004; Bové 2006). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. asiaticus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, but none of these species have been recorded on the Rutaceae (DEWHA 2009).
- In the absence of a suitable vector the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Distribution of the imported commodity in the PRA area***

- The imported budwood would be distributed to multiple destinations throughout Australia for propagation. Distribution of infected budwood commercially would facilitate the distribution of '*Ca*. L. asiaticus'.
- Plants grafted with imported infected budwood may be distributed to greenhouses or retail shops where the bacterium may continue multiplying within the host. Grafted plants are unlikely to be grown in isolation, providing greater opportunity for this bacterium to move to a suitable host. However, in the absence of a suitable vector, the bacterium itself is unable to move from infected plants to other hosts.
- Asymptomatic plants resulting from grafting with imported budwood may also be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter asiaticus' would need to survive transportation and storage within the PRA area. Grafted plants are expected to be maintained at moderate

temperatures and humidity levels to ensure their survival, so a high portion of infected plants are likely to reach areas of host abundance.

#### *Risks from by-products and waste*

- Although the intended use of budwood is for propagation, and all imported material is likely to be used, waste material may be generated. Whole or parts of the budwood may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem-limited bacteria.
- Although '*Ca*. L. asiaticus' is considered heat-tolerant (Bové 2006; Lopes *et al*. 2009b), composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. asiaticus' is heat-sensitive to temperatures above 38 °C (Lopes *et al*. 2009a).
- A proportion of garden waste would be managed through green waste centres. Unlike managed waste, garden waste is more likely to be retained in the urban and peri-urban environment for a period of time before being disposed of at green waste centres.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. However, acquisition and transmission periods for *D. citri* vary from 1–25 days from living host tissue (Halbert and Manjunath 2004).

The association of '*Ca*. L. asiaticus' with budwood and the likely distribution of infected budwood and grafted plants support an assessment of 'high' for the distribution of this species in budwood.

## Pathway 4—Fresh leaves

### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia with the trade in fresh leaves from countries where the pathogen is present is **MODERATE**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter species' can be associated with all vegetative parts of host plants including foliage (Tatineni *et al.* 2008), so fresh leaves of host plants can be infected and provide a pathway for the importation of the bacterium into Australia.
- '*Candidatus* Liberibacter asiaticus' survives within the phloem vascular system of the host plants (Bové 2006; Kim *et al*. 2009). Although the bacterium infects phloem, the infection has an uneven distribution within the host tissues (Huang 1979) and may not necessarily be present in the leaves of infected trees.
- '*Candidatus* Liberibacter asiaticus' is an aggressive pathogen and is able to produce severe symptoms under favourable conditions (da Graça 1991). Symptom expression is influenced by temperature and varies with season; in unfavourable environments symptoms may not be expressed (Bové 2006).
- Initial symptoms caused by '*Ca*. L. asiaticus' are difficult to distinguish from nutrient deficiencies or other plant diseases (Li *et al*. 2009). Typical foliage symptoms include pale yellowing of leaves and blotchy mottle (Kim *et al*. 2009). Infected leaves can become upright, followed by leaf drop at the laminar abscission zone and twig dieback

at a later stage (Kim *et al*. 2009). Therefore, symptomatic leaves would most likely be detected on arrival in Australia.
- However, infected plants may be asymptomatic in the initial stages of infection and can be easily overlooked.
- '*Candidatus* Liberibacter asiaticus' has a long incubation period and many infected plants remain symptomless, particularly those which are newly infected (Xu *et al*. 1988b; Wang *et al*. 1996; Gottwald *et al*. 2007). It is likely that asymptomatic leaves infected by '*Ca.* L. asiaticus' would pass routine visual inspections.

***Ability of the pest to survive transport and storage***
- Fresh leaves destined for Australia would be air freighted and, during transportation and storage, kept at low temperatures and high humidity to ensure the material remains fresh. '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008). For example, long-term storage of plant samples at 4 ºC for two months did not affect the detection of '*Ca*. L. asiaticus' (Li *et al*. 2008). Although the viability of the bacteria was not confirmed, Li *et al*. (2008) demonstrated that over a 10 week period of testing the quantity of bacterium in plant samples did not change, which may indicate the presence of some viable bacteria.

***Ability of the pest to survive existing pest management procedures***
- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated (Floyd and Krass 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

'*Candidatus* Liberibacter asiaticus' is associated with fresh leaves, the foliage of infected plants may remain asymptomatic and the bacterium is likely to remain viable during transport and storage. However, the bacterium is unevenly distributed within host trees and may not be present in leaves sourced from infected hosts. Therefore an assessment of 'moderate' for the importation of this species in fresh leaves is allocated.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed within Australia in a viable state with imported fresh leaves from countries where the pathogen is present and transferred to a suitable host is **EXTREMELY LOW**.

***Ability of the pest to move from the pathway to a suitable host***
- '*Candidatus* Liberibacter asiaticus' is unable to move independently from imported fresh leaves to a suitable host. The bacterium is able to move to a suitable host only by grafting or by psyllid vectors (Gottwald *et al*. 2007). Cut leaves are not suitable for grafting.
- Without grafting, the transfer of '*Ca.* L. asiaticus' from imported fresh leaves to a suitable host would require a vector. '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. asiaticus' is vectored by *Trioza erytreae* and *Diaphorina citri* (Aubert 2008). These specific psyllid vectors are not present in Australia.
- Although five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) and one species of *Diaphorina* (*D. tryoni*) are present in Australia, none of these species have been recorded on the Rutaceae (DEWHA 2009).

- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- Furthermore, even if known psyllid vectors are present in Australia, they preferentially feed on new flushes of growth (Gottwald *et al*. 2007) and are unlikely to feed on cut leaves.

***Distribution of the imported commodity in the PRA area***

- Asymptomatic leaves will be distributed for all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption (e.g. curry leaves).
- Fresh leaves may be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the produce is likely to reach areas of host abundance.
- '*Candidatus* Liberibacter asiaticus' would need to survive transportation and storage within the PRA area. Fresh leaves would be kept at low temperatures and high humidity to ensure the material remains fresh. '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008). Thus, storage conditions are unlikely to have any large impact on the level of '*Ca.* L. asiaticus' infection in fresh leaves distributed for retail or market sale.

***Risks from by-products and waste***

- Although the intended use of fresh leaves includes all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption, waste material would be generated. Fresh leaves may be disposed of at multiple locations throughout Australia in compost bins or amongst general waste.
- '*Candidatus* Liberibacter species' have been detected in all vegetative parts of the host including roots, stem, bark and leaves (Tatineni *et al*. 2008; Li *et al*. 2009). Any discarded waste containing this bacterium would be colonised by saprophytic microorganisms that can tolerate a wide range of conditions (Lynch and Hobbie 1988) and are likely to outcompete a phloem-limited bacteria.
- Although '*Ca*. L. asiaticus' is considered heat-tolerant (Bové 2006; Lopes *et al*. 2009b), composting waste material is likely to generate high temperatures that can be lethal to a range of pathogens (Noble and Roberts 2004), and '*Ca*. L. asiaticus' is heat-sensitive to temperatures above 38 °C (Lopes *et al*. 2009a).
- A relatively high proportion of household or commercial waste would be managed through regulated refuse collection and disposal services. Managed waste will remove '*Ca*. L. species' from the household and environment, reducing the likelihood that susceptible plants will be exposed to bacteria.
- Even if psyllid vectors are present in Australia, they preferentially feed on new flush and are unlikely to feed on waste material. If an insect vector does feed on discarded material it may acquire the bacterium and spread this bacterium to other hosts. Acquisition and transmission times for *D. citri* vary from 1–25 days (Halbert and Manjunath 2004).

Although '*Ca.* L. asiaticus' is associated with fresh leaves, the bacterium is unable to disperse unassisted, is unlikely to be transmitted by a vector even if known vectors are present in Australia (as vectors prefer to feed on fresh foliage) and the pathway is unsuitable for graft transmission. Therefore an assessment of 'extremely low' is given for the distribution of this species in fresh leaves.

## Pathway 5—Seed for sowing

### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia with trade in seed of Rutaceous species for sowing from countries where the pathogen is present is **LOW**.

#### *Association of the pest with the pathway*

- '*Candidatus* Liberibacter asiaticus' has been detected in citrus seed coats but not in the seed endosperm and embryo (Li *et al*. 2008; Tatineni *et al*. 2008). Therefore this bacterium is associated with the pathway.
- '*Candidatus* Liberibacter asiaticus' is also associated experimentally with seeds of dodder and periwinkle (Benyon *et al*. 2008).
- Seeds from '*Ca.* L. species' affected trees often show various degrees of abortion (Bové 2006), probably related to the presence of the pathogens in the seed coats.
- Based on research into seed to seedling transmission at low levels (Benyon *et al*. 2008; Graham *et al*. 2008; Hartung *et al*. 2008; Shatters 2008), the USA banned the importation of seed for sowing from species of known host genera in the family Rutaceae from countries where citrus infecting '*Ca.* L. species' are present (APHIS 2008b).
- A proportion of fruit on infected trees is aborted and the remaining fruit has a high rate of aborted seed (Halbert and Manjunath 2004). Un-aborted seed in infected fruits are characteristically small and brownish/black in colour (Bové 2006). Such seeds may be rejected due to quality issues.
- The distribution of '*Ca.* L. asiaticus' within the host tissues is variable, with the level of infection in symptomatic fruit being comparatively low (Li *et al*. 2009).
- Asymptomatic fruit, harvested from symptomatic branches, have tested positive for the bacteria (Li *et al*. 2009). The detection of '*Ca*. L. species' in fruit harvested from asymptomatic branches has not been recorded. Based on this knowledge, the level and incidence of bacterial infection of seed from asymptomatic fruit, particularly from healthy trees, is likely to be low.

#### *Ability of the pest to survive transport and storage*

- Seed would be shipped in refrigerated containers maintained at 3–7 ºC (Willits and Newcomb Inc 2004). '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008).
- Long-term storage of plant samples at 4 ºC for two months did not affect the detection of '*Ca*. L. asiaticus' (Li *et al*. 2008). Although the viability of the bacteria was not confirmed, Li *et al*. (2008) demonstrated that over a 10 week period of testing the quantity of bacterium in plant samples did not change, which may indicate the presence of some viable bacteria. Therefore, storage conditions are unlikely to have any impact on the survival of '*Ca.* L. asiaticus' bacteria in imported seeds.
- Citrus seed is highly perishable and sowing should occur as soon as possible following importation. Temperature extremes and exposure to direct sunlight may be fatal to citrus seeds (Willits and Newcomb Inc 2004). Thus, the length of time taken to transport citrus seed from the country of origin, and the time it spends in storage prior to export, may lower its viability and that of any associated bacterium.

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated (Floyd and Krass 2006).
- Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

The lack of fruit produced by infected trees, the high rate of aborted seeds and the discolouration of the seeds supports an assessment of 'low' for the importation of '*Ca*. L. asiaticus' in seed.

## Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed within Australia with imported seed from countries where the pathogen is present is **MODERATE**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter asiaticus' arriving in Australia with imported seed for sowing may not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host. '*Candidatus* Liberibacter species' can be detected at a high level in the seed coats (Tatineni *et al*. 2008).
- Unlike seed imported in fruit, seed for sowing is specifically imported for the purpose of propagation and can be a significant investment for importers. Infected seed is therefore likely to be sown directly into suitable habitats, to maximise survival, at multiple locations in Australia. Furthermore, considerable resources will be used to ensure the health and survival of the imported seed.
- Seed to seedling transmission has been reported when seed sourced from infected plants were grown (Tirtawidjaja 1981; Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008). However, the infection rate can decrease over time and symptom expression can be lost completely (Benyon *et al*. 2008; Graham *et al*. 2008; Shatters 2008).
- Recent experimental studies have confirmed the seed to seedling transmission of '*Ca*. L. asiaticus' (Benyon *et al*. 2009). However, over a three year period, the bacterial titre remained at very low levels and most, if not all, of the '*Ca*. L. asiaticus' positive seedlings did not developed typical HLB symptoms (Benyon *et al*. 2009).
- There is no report of seed transmission in *Murraya* species or any other citrus relative. However, seed transmission (as high as 53%) of '*Ca*. L. asiaticus' was recently reported experimentally for dodder and periwinkle (Zhou *et al*. 2008b).
- If an infected seedling does establish, insect vectors would be required to feed to move the bacterium to new hosts. In the absence of a vector, the bacterium is unable to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).
- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Bové 2006, Halbert and Manjunath 2004). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species). However, in the absence of psyllid vectors the bacterium is unable to move to these available hosts.

***Distribution of the imported commodity in the PRA area***

- The distribution of seed for sowing would be for retail distribution to multiple destinations throughout Australia. Seed for sowing would also be distributed to multiple locations for propagation.
- Seeds would be imported to introduce new genetic material to commercial citrus production. Imported citrus seeds are likely to be distributed to suitable areas where citrus is grown.
- Distribution of infected seed commercially through nurseries would facilitate the distribution of '*Ca*. L. asiaticus'. Asymptomatic plants that develop from infected seed may also be overlooked and sold to commercial users and households.
- '*Candidatus* Liberibacter asiaticus' would need to survive transportation and storage within the PRA area. '*Candidatus* Liberibacter asiaticus' is able to survive at low, as well as at high, temperatures in the host tissues (Bové 2006; Li *et al*. 2008). Thus, transport and storage conditions are unlikely to have any large impact on the level of '*Ca.* L. asiaticus' infection in seed distributed for sowing.

***Risks from by-products and waste***

- The intended use of seed is for propagation, and all imported seed would be grown under ideal conditions. Seed that does not survive transportation and storage may shrivel and discolour, and may not be used for sowing. Therefore, waste material may be generated. As the bacterium can infect the entire plant (Tatineni *et al*. 2008) any such material that is discarded may contain the bacterium.
- Seeds of most *Citrus* species desiccate quite quickly when exposed to warm air temperatures, rapidly reducing the moisture content of the seed (Barton 1943; Mumford and Panggabean 1982; Edwards and Mumford 1985). As a consequence, discarded seeds may not germinate because they do not tolerate moisture loss.
- If a seed does germinate and seed to seedling transmission occurs, a systemically infected plant will establish; however, the infection is transient and may be lost over time (Shatter 2008).
- The transfer of '*Ca.* L. asiaticus' from an infected seedling established at a waste depot or in the backyard to a host would require a vector. The only known vectors are not present in Australia.
- Since '*Ca*. L. species' are spread only by vectors or grafting, it is highly likely that an infected plant, which develops in an area where the psyllid vector is not known to occur, will represent an isolated infection (Floyd and Krass 2006).

Although, '*Ca.* L. species' are associated with seeds at variable rates, the low level of seed to seedling transmission and loss of symptoms over time, support an assessment of 'moderate' for the distribution of '*Ca.* L. asiaticus' in seeds.

## Pathway 6—Infected Psyllid

### Probability of importation

The likelihood that '*Candidatus* Liberibacter asiaticus' will arrive in Australia in infected psyllid vectors (*Diaphorina citri*)[10] from countries where the pathogen is present is **MODERATE**.

[10] Although both *Diaphorina citri* and *Trioza erytreae* are reported to vector '*Ca.* L. asiaticus' in Réunion (Aubert 2008), only *D. citri* is considered here as it is the primary vector.

***Association of the pest with the pathway***

- '*Candidatus* Liberibacter asiaticus' is naturally associated with *Diaphorina citri* (Capoor *et al*. 1974; Xu *et al*. 1988a). Therefore this bacterium is associated with the pathway.
- *Diaphorina citri* acquires the bacterium while feeding on an infected plant (Bové 2006; Lopes *et al*. 2009b). Once the psyllid has acquired the bacterium from an infected host, the psyllid maintains the bacteria and the ability to transmit the disease throughout its life (Gottwald *et al.* 2007).
- *Diaphorina citri* is an efficient vector of '*Ca*. L. asiaticus'. The bacterium has been detected in second, third, fourth and fifth instars nymphs and adults (Capoor *et al*. 1974; Xu *et al*. 1988a; Hung *et al*. 2004). Therefore, not only the adults but also the juvenile stages of *D. citri* have the potential to be infected with '*Ca*. L. asiaticus'.
- The level of '*Ca*. L. asiaticus' infection in *D. citri* has been found to vary depending on the life stage at which the psyllid fed on infected plants. Psyllids that completed their development on HLB infected trees were more likely to carry the pathogen than psyllids that fed on HLB infected trees as adults only (Roger *et al*. 2008; Xu *et al*. 1988a).
  - '*Candidatus* Liberibacter asiaticus' was detectable in 20–30% of psyllids that fed on plants as adults only, whereas up to 100% of the psyllid adults that developed from nymphs on infected plant material tested positive for the bacterium (Roger *et al*. 2008).
  - Adults that developed from infected nymphs were positive for the bacterium and were able to transmit the bacterium immediately (Xu *et al*. 1988a).
- Seasonal levels of '*Ca*. L. asiaticus' in psyllid populations have been studied in Japan (Sadoyama and Takushi 2008) and Florida (Manjunath *et al*. 2008). These studies indicate that seasonal titres of bacteria fluctuate in susceptible host plants, and consequently in the vectors, falling in summer due to high leaf temperatures, when ambient temperatures and solar radiation are high, saturation deficits low, and relative humidity is high (Manjunath *et al*. 2008).
- About 40% of adult *D. citri* collected in the autumn from Malaysia, and 1% of the psyllids collected in the winter from India, were found to carry '*Ca*. L. asiaticus' (Bové *et al.* 1993). In Florida, an increase in '*Ca*. L. asiaticus' titre has been noticed in plants in the autumn (Irey 2006; Irey *et al.* 2006). These results suggest a higher titre of '*Ca*. L. asiaticus' in both plants and insects in the autumn rather than in the winter.
- *Diaphorina citri* adults and nymphs have been collected from infected *Murraya* species in Florida. About 17% of the psyllid adults and 12% of the nymphs samples tested were positive for '*Ca*. L. asiaticus' (Ramadugu *et al*. 2008).
- Variation in the level of '*Ca*. L. asiaticus' infection across the different biotypes of *D. citri* have also been reported (Su 2008). The Taiwanese biotype of *D. citri* is a less efficient vector of HLB (Su 2008). Less than 5% of adults were found to carry the HLB pathogen after acquisition feeding on diseased citrus plants over one day, while the nymphs acquired the pathogen at much higher rate than adults (Su 2008).
- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004; Aubert 1987a). It would be possible for infected psyllids to enter Australia via trade in commodities, such as fresh fruit, fresh leaves, nursery stock and budwood of suitable host species (Refer to Chapter 5.4). Infective *D. citri* are known to move on leaf and twig material and fruit (Halbert and Manjunath 2004).
- Leaf material or trash in imported fruit is a significant risk for the entry of all life stages of *D. citri* as eggs are laid on the leaves (Grafton-Cardwell *et al*. 2006) and

nymphs and adults feed under the surfaces of leaves (Shivankar *et al*. 2000; CABI 2007).
- Related species *Diaphorina communis* and *D. murrayi* have been reported on citrus from India (Halbert and Manjunath 2004) where '*Ca*. L. asiaticus' is present. However, these psyllids have not been reported to vector the bacterium (Halbert and Manjunath 2004).

***Ability of the pest to survive transport and storage***
- Once infected with '*Ca.* L. species' psyllids remain infective for life (Gottwald *et al.* 2007). '*Candidatus* Liberibacter asiaticus' is therefore able to survive for long periods in infective psyllids provided the conditions are suitable for the survival of the psyllids.
- Infected psyllids are likely to enter Australia via trade in fresh fruit, fresh leaves, nursery stock and budwood of suitable host species (Refer to Chapter 5.4) and must survive the transport and storage conditions for these commodities in order to vector '*Ca.* L. asiaticus'.
- Adult *D. citri* are able to survive up to 52.5–94.5 hours without feeding if suitable foliage is not available (McFarland and Hoy 2001).
- Infected psyllids associated with fresh leaves or nursery stock has the potential to continue feeding during transport and storage. Adult *D. citri* may survive for 8–9 months over winter provided suitable foliage is available (Yang *et al*. 2006).
- The threshold temperature for nymphal development is 10–11 °C (Liu and Tsai 2000) and the commodities they are likely to be in association with will be shipped at low (4–6 °C for fruit) to moderate temperatures.
- Transport temperatures are not lethal to *D. citri* as the psyllid is able to survive much lower temperatures (Hall 2008b). *Diaphorina citri* tolerate low ambient temperatures (e.g. mid winter average daily temperatures between 5.4–6.9 °C, with minimums from -5.3 to -7.5 °C (Xie *et al.* 1988)). These conditions are likely to stop bacterial multiplication, but are considered unlikely to have any impact on the survival of '*Ca*. L. asiaticus' within the psyllid.
- There is no reason to suspect that the duration of transport and storage would lower the viability of '*Ca*. L. asiaticus' in *D. citri*.
- There have been 170 interceptions of live *D. citri* at ports in the USA, on plant material with an Asian origin, from 1985–2003 (Halbert and Manjunath 2004; Sullivan and Zink 2007). There are an additional 73 records of interceptions of live *Diaphorina* species on Rutaceous plants from Asia (Halbert and Manjunath 2004). This indicates that *D. citri* would survive during transport.
- Live *D. citri* have been repeatedly intercepted on citrus fruit from the Bahamas and shipped to Florida for processing (Halbert and Núñez 2004; Sullivan and Zink 2007). In addition, *D. citri* has been intercepted from Belize on citrus in baggage at Houston Airport, Texas (Halbert and Núñez 2004). It has also been detected on citrus fruit from Mexico into Texas (Halbert and Núñez 2004).
- Live *D. citri* were intercepted in shipments of fresh curry leaves (*M. koenigii*) from Hawaii to California (Wilkinson 2007; Filippini 2008). Recently, about 100 live nymphs and adults were detected on fresh curry leaves in a package from Texas to California (CDFA 2009; The Packer 2009).
- Live nymphs and adults of *D. citri* were detected on fresh curry leaves in unaccompanied baggage from India to California in July 2009. Subsequent testing confirmed the presence of the HLB pathogen in one of the insects (CDFA 2009; Associated Press 2009).

***Ability of the pest to survive existing pest management procedures***

- '*Candidatus* Liberibacter species' are typically managed by chemical control of vector populations (Lopes *et al*. 2007; Lopes *et al*. 2009b) and the removal and destruction of infected trees to reduce inoculum, since the bacterium is systemic and cannot be eliminated (Floyd and Krass 2006).
- Insecticides, neem oil, petroleum spray oil and biological control agents may be used in orchard management of psyllids (Aubert and Quilici 1984; Xu *et al*. 1991; Chakravarthi *et al*. 1998; Rae *et al*. 1997). These control methods are likely to reduce, but not necessarily eliminate, infected *D. citri*. The methods will have no impact on the level of '*Ca.* L. asiaticus' infection in surviving psyllids.

The ability of '*Ca.* L. asiaticus' to infect *Diaphorina citri* persistently, association of the psyllid with host plants, variable number of carriers and the likelihood of the bacterium remaining viable within the psyllid during transport and storage supports an assessment of 'moderate'.

### Probability of distribution

The likelihood that '*Candidatus* Liberibacter asiaticus' will be distributed within Australia with infected psyllid to suitable hosts from countries where the pathogen is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- '*Candidatus* Liberibacter asiaticus' arriving in Australia with infected *D. citri* will continue multiplying inside the psyllid vector (Manjunath *et* al. 2008).
- Adults and fourth and fifth instar nymphs are able to transmit '*Ca*. L. asiaticus' (Halbert and Manjunath 2004). '*Candidatus* Liberibacter asiaticus' has also been detected in third instar nymphs of *D. citri* (Hung *et al*. 2004).
- Sessile nymphs infected with '*Ca.* L. species' are known to retain the bacterium on maturing to adults (Halbert and Manjunath 2004).
- Immature stages of the psyllid must have suitable food sources to allow them to survive and develop. Due to the relatively long developmental time—up to 48 days from egg to adult (Halbert and Manjunath 2004; EPPO 2009), there is likely to be some mortality of eggs and nymphs.
- For '*Ca.* L. asiaticus' to be transferred from the psyllid to a plant host, infected psyllids would need to find a host to feed on and successfully transmit the bacterium to the host.
- Adults of *D. citri* have been recorded to regularly fly distances of 8–60 m (Hall 2008a), and to travel 0.5–1 km in wind drifts (Barkley and Beattie 2008), and are able to survive up to 52.5–94.5 hours without feeding if suitable foliage is not available (McFarland and Hoy 2001). Therefore *D. citri* carrying '*Ca*. L. asiaticus' are capable of locating potential host plants to transmit the bacterium.
- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Bové 2006, Halbert and Manjunath 2004). *Diaphorina citri* feed and reproduce on the Rutaceae, including cultivated *Citrus* species and Australian naturalised/native species *Murraya paniculata* and *Citrus australasica* (Halbert and Manjunath 2004). There is significant overlap between the host range of the bacterium and the psyllid suggesting the psyllid is likely to distribute the bacterium to a suitable host.
- Suitable hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

- Once a suitable host has been found, infected psyllids are able to transmit the pathogen (Gottwald *et al.* 2007). However, the rate of transmission has not been recorded directly.

***Distribution of the imported commodity in the PRA area***

- Psyllids carrying '*Ca.* L. asiaticus' imported into Australia on fruit, fresh leaves, budwood and/or nursery stock of host plants may be distributed throughout the PRA area with these commodities as the commodities are imported for wholesale and retail sale to multiple locations.
- Infected *D. citri* would need to survive transportation and storage within the PRA area. The threshold temperature for nymphal development is 10–11 °C (Liu and Tsai 2000) and '*Ca.* L. asiaticus' is able to survive at low, as well as at high, temperatures (Bové 2006; Li *et al*. 2008).
- *Diaphorina citri* survives in a wide range of temperatures extremes, from 45 °C in Saudi Arabia to -7 °C or -8 °C in subtropical China (Aubert 1988, 1990) and is therefore likely to survive transport and storage temperatures. It is considered unlikely that transport and storage conditions would reduce or eliminate '*Ca*. L. asiaticus' within the psyllid.

***Risks from by-products and waste***

- Infected psyllids are likely to be distributed throughout the PRA area with fresh fruit, fresh leaves, nursery stock or budwood of Rutaceous hosts. Although the intended use of these commodities is for human consumption (fruit, fresh leaves) and propagation (nursery stock, budwood), waste material would be generated. Whole or parts of the imported fruit, fresh leaves, nursery stock or budwood infested or contaminated with life stages of the psyllid may be disposed of at multiple locations throughout Australia in compost bins, amongst general household waste or green waste.
- Infected psyllids may survive on waste material for a short time before dispersing to suitable hosts. *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to fly significant distances autonomously (Barkley and Beattie 2008).

The association of '*Ca*. L. asiaticus' with *Diaphorina citri*, the ability for infected psyllids to disperse both independently and through the movement of infested host commodities, the host range overlap of the two species, and the presence of multiple hosts within the PRA area, support an assessment of 'high' for the distribution of '*Ca*. L. asiaticus' in *Diaphorina citri*.

## Overall probability of entry (importation x distribution)

The overall probability of entry '*Ca*. L. asiaticus' is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2). The overall probability of entry for the six pathways being assessed in this PRA is set out in Table 5.7.

**Table 5.7: Overall probability of entry of '*Ca*. L. asiaticus' on different pathways**

| Pathway | Probability of importation | Probability of distribution | Overall probability of entry |
|---|---|---|---|
| Fruit (containing seed) | Very low | Extremely low | Extremely low |
| Nursery stock (live plants) | High | High | High |
| Budwood | Moderate | High | Moderate |
| Fresh leaves | Moderate | Extremely low | Extremely low |
| Seed for sowing | Low | Moderate | Low |
| Infected psyllids | Moderate | High | Moderate |

### 5.3.2 Probability of establishment

The likelihood that '*Candidatus* Liberibacter asiaticus' will establish within Australia, based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is **HIGH**.

***Availability of suitable hosts, alternative hosts and vectors in the PRA area***

- Rutaceous plants are the natural hosts of '*Ca*. L. asiaticus' with all species and cultivars of citrus susceptible to infection (Bové 2006; Halbert and Manjunath 2004). These hosts are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- *Diaphorina citri* was detected in the Northern Territory in 1915, but was eliminated during the 1918–1922 citrus canker eradication programs, and there have been no detections of this pest since (Bellis *et al.* 2005). There has never been a detection of HLB in Australia (Bellis *et al.* 2005).
- Although one species of *Diaphorina* (*D. tryoni*) is present in Australia, this species has not been recorded on the Rutaceae (DEWHA 2009).
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to transmit this bacterium.
- In the absence of a vector, the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the bacteria may fail to persist in the environment (Floyd and Krass 2006).

***Suitability of the environment***

- '*Candidatus* Liberibacter asiaticus' has established successfully in the citrus growing regions of its range and has been reported from Bangladesh, Bhutan, Brazil (Sao Paulo, Minas Gerais and Parana states), Cambodia, China, Cuba, Dominican Republic, East Timor, India, Indonesia, Japan, Laos, Malaysia, Mauritius, Mexico, Myanmar, Nepal, Pakistan, Papua New Guinea, Philippines, Puerto Rico, Réunion, Saudi Arabia, Sri Lanka, Taiwan, Thailand, USA (Florida, Georgia, Louisiana and South Carolina states), Vietnam and Yemen (Teixeira *et al.* 2005a, 2005b; Bové 2006; da Graça 2008; Martínez *et al*. 2008; Matos *et al*. 2009; NAPPO 2009a, 2009b).
- The current reported distribution of '*Ca.* L. asiaticus' suggests that there are similar environments in parts of Australia (Peel *et al.* 2007) that would be suitable for its establishment.
- '*Candidatus* Liberibacter asiaticus' is inactivated at higher temperatures (Cheema *et al*. 1982; Huang 1978; Song *et al*. 1999; Zhao 1981) and such inactivation appears to influence the severity of the disease in parts of Asia, where rates of decline of trees appear to be slower in Pakistan, for example, than in the cooler parts of Southeast Asia (Beattie and Barkley 2009).
- Both '*Ca.* L. asiaticus' and its natural vector *D. citri* are heat-tolerant, relative to '*Ca.* L. africanus' and its vector *T. erytreae* (Bové 2006), and the Australian environment is suitable for both the pathogen and the vector.
  - Climate modelling for Australia has determined that all major citrus growing regions would be favourable for the establishment of '*Ca*. L. asiaticus'. Results demonstrated that high summer temperatures, which occasionally exceed 40 °C, will not be high enough to limit the distribution of '*Ca*. L. asiaticus' in tree canopies (Barkley and Beattie 2008). Additionally, the lowest temperatures

recorded in the citrus growing regions of Australia are unlikely to prevent the establishment of this bacterium (Barkley and Beattie 2008).
- *Diaphorina citri* is better adapted to regions with high temperatures and low relative humidity than to regions with medium to high temperatures and high relative humidity (Halbert and Manjunath 2004). Therefore this species will survive in most of the citrus growing regions of Australia.
- Climate modelling has predicted that all major citrus growing regions of Australia would have suitable climates favourable for *Diaphorina citri* (Barkley and Beattie 2008).

***The reproductive strategy and survival of the pest***

- '*Candidatus* Liberibacter asiaticus' multiplies and survives in the phloem of infected host plants and in its vector, *D. citri* (Bové 2006; Manjunath *et al* 2008).
- '*Candidatus* Liberibacter asiaticus' is reliant on infected host plants for survival and will survive as long as the infected plant survives. Symptom development may be delayed for several months, or may take 2–3 years after initial infection (Capoor *et al*. 1974; Hung *et al*. 2001; Gottwald *et al*. 2007).
- The survival and multiplication of this bacterium within its host is influenced by temperature. '*Candidatus* Liberibacter asiaticus' is heat tolerant and is established across a wide temperature range (Bové 2006; Gasparoto *et al* 2008; Lopes *et al*. 2009a).
  - Plants graft-inoculated with '*Ca.* L. asiaticus' from India and Philippines, both produced symptoms at 22–24 °C and at 27–32 °C. This experiment, as well as the field observations, shows that HLB in Asia is heat-tolerant and symptoms occur even when temperatures are well above 30 °C (Bové 2006).
  - Trees naturally infected with '*Ca.* L. asiaticus' from Brazil produced symptoms at 22–24 °C and at 27–32 °C. Multiplication of the bacterium in infected plants was not affected by temperatures up to 35 °C, but at 38 °C multiplication of '*Ca.* L. asiaticus' was drastically reduced (Lopes *at al*. 2009a). This experiment shows that '*Ca.* L. asiaticus' is heat-tolerant to temperatures up to 35 °C (Lopes *et al*. 2009a).
  - These studies, along with the known tolerance of *D. citri* to heat, explain why '*Ca.* L. asiaticus' occurs in hot environments (Lopes *et al*. 2009a).
- In the sieve tubes of leaves of citrus varieties, '*Ca*. L. asiaticus' had a tendency to multiply most abundantly in summer and slightly less in autumn and winter; however, the bacterium was detected all year round (Su 2008).
- '*Candidatus* Liberibacter asiaticus' may also survive as different strains. Strains of '*Ca*. L. asiaticus' have been reported from India and Taiwan (da Graça 1991; Tsai *et al*. 2008).
  - In India different strains showed varying degrees of virulence when grafted onto a common host. These strains were grouped as mild, severe and very severe (da Graça 1991).
  - Monoclonal antibodies raised against the bacterium from Poona, India, failed to detect HLB bacterium from China, Thailand, Malaysia and other parts of India (da Graça 1991).
  - Based on pathogenicity and virulence tests on a citrus cultivar set, four strains of '*Ca*. L. asiaticus' have been identified: Strain I showed pathogenicity on mandarin and sweet orange by inducing typical HLB symptoms; Strain II showed high virulence on all differential cultivars and multiplied fast in all cultivars; Strain III caused intermediate symptoms on mandarin and sweet orange and mild symptoms on pomelo and did not infect Eureka lemon; and a mild Strain IV, which infected

  mandarin and sweet orange without causing symptoms, and was rarely isolated (Tsai *et al*. 2008).
  - Huanglongbing in India is reported to be more severe on mandarins and sweet orange, while lemons, limes, grapefruit, and pomelos are more tolerant (da Graça 1991). However, other strains of Asian HLB in Taiwan and those found recently in Florida affect all commercial citrus species severely (Gottwald *et al.* 2007). It is possible that strains of the HLB pathogen can adapt to citrus species and cultivars over time and may explain why grapefruit and pomelo were at first considered resistant in areas such as Taiwan but are now considered quite susceptible to HLB (Gottwald *et al.* 2007).
- Outside a host plant, '*Ca.* L. asiaticus' can infect nymphs and adult citrus psyllids (Bové 2006). Once infected with '*Ca.* L. species' the psyllids remain infective for life (Gottwald *et al.* 2007) and thus the bacterium will survive for as long as the vector is alive. The duration of the lifecycle of *D. citri* from egg to adult is up to 47 days depending upon food supply and ambient temperature (Knapp *et al*. 2006); and adults are known to survive 8–9 months over winter on suitable hosts (Yang *et al*. 2006). '*Candidatus* Liberibacter asiaticus' is therefore able to survive for long periods in infective psyllids.

***Cultural practices and control measures***

- There are currently no control measures in commercial orchards that target '*Ca* L. asiaticus' and no programs exist for garden, amenity or native hosts. Therefore current control measures are unlikely to impact on the establishment of '*Ca* L. asiaticus' in Australia.
- '*Candidatus* Liberibacter asiaticus' is difficult to manage due to the nonspecific nature of disease symptoms, prolonged latency of the disease in field trees, probable irregular distribution of the pathogen in trees, the effect of the environment (especially temperature) on symptom expression and possibly on bacterial multiplication, probable variations in tolerances to the bacterium in both the plant host and the vectors, and the fastidious nature of the bacterium (Manjunath *et al*. 2008).
- There is no cure for '*Ca*. L. asiaticus' infected trees, which decline and may senesce within a few years of infection (Polek *et al.* 2007), although symptoms abate temporarily when trees are injected with antibiotics (Halbert and Manjunath 2004).
- In the early 1990's, a multinational rehabilitation project was instituted by the Food and Agriculture Organisation of the United Nations (FAO) in southeast Asian citrus regions to develop practical control strategies against '*Ca.* L. asiaticus'. Recommended management strategies included geographical isolation, disease certification programs for budwood sources, insect proof screens and propagation houses, control of insect vectors and commercial plantation removal of infected trees to reduce inoculum sources (Gottwald *et al*. 2007). These methods are likely to impact on the establishment of '*Ca.* L. asiaticus' in Australia should they be adopted.
- Eradication may be possible if the bacterium is detected early and in the absence of psyllid vectors. However, there are no cases of successful eradication programs for '*Ca*. L. asiaticus' (Polek *et al.* 2007).
- Since the bacterium cannot be eliminated, infected trees are removed and destroyed (Floyd and Krass 2006). Treatment with antibiotics suppresses the bacterium symptoms; however, it is impractical, phytotoxic, and will not eliminate the bacteria from the tree (Floyd and Krass 2006).

'*Candidatus* Liberibacter asiaticus' is able to survive and multiply within a range of common hosts and its psyllid vectors, and survives in diverse climatic conditions that would cover most of Australia. Therefore an assessment of 'high' is given for the establishment of '*Ca.* L. asiaticus' in Australia.

### 5.3.3 Probability of spread

The likelihood that '*Candidatus* Liberibacter asiaticus' will spread based on a comparison of factors in the area of origin and in Australia that affect the expansion of the geographic distribution of the pest is **HIGH (VERY LOW[11])**.

***The suitability of the natural or managed environment for natural spread***

- '*Candidatus* Liberibacter asiaticus' may have originated in India (Halbert and Manjunath 2004). From India, '*Ca* L. asiaticus' spread through Asia, initially to mainland China (probably in the early 1930s), then to Taiwan, Indonesia, the Philippines, Malaysia, Thailand, Vietnam, Japan and Papua New Guinea (Weinert *et al*. 2004; da Graça 2008; Beattie *et al*. 2008).
- '*Candidatus* Liberibacter asiaticus' was first discovered in Iriomote Island, Japan in 1988 (Miyakawa and Tsuno 1989). Since this time the bacterium has spread north up to Tokuno-shima Island, Kagoshima Prefecture in 2003 (Urasaki *et al*. 2008).
- In the Americas, '*Ca.* L. asiaticus' was first reported in 2005 from Brazil and has spread to Cuba, the Dominican Republic, Puerto Rico, Mexico and the USA (Florida, Georgia, Louisiana and South Carolina states) (Teixeira *et al*. 2005a, 2005b; CDFA 2008a; APHIS 2008a; Matos *et al*. 2009; NAPPO 2009b). There are similarities in the natural and urban environments of these areas with those in Australia, which suggests that '*Ca*. L. asiaticus' could spread in Australia (Peel *et al.* 2007).
- The continued spread of '*Ca.* L. asiaticus' has caused it to become a serious constraint for the citrus industry worldwide (Su 2008).
- Host plants that support the spread of '*Ca*. L. asiaticus' are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment. Hosts include all species and cultivars of citrus and some non-citrus Rutaceae (Halbert and Manjunath 2004; Bové 2006).
- Natural spread of '*Ca.* L. species' is dependent on the transmission of the bacterium by its psyllid vectors (Rogers *et al.* 2008). The spread of '*Ca.* L. asiaticus' within Australia would rely on *Diaphorina citri* (da Graça 2008). Natural spread in the absence of vectors is limited or absent (Polek *et al.* 2007).
- In the absence of vectors, spread of '*Ca.* L. species' would rely on the movement of infective propagative material (Polek *et al.* 2007). However, the bacterium is unable to move from infected plants to new host plants; infected plants will ultimately senesce and the pathogen may fail to persist in the environment (Floyd and Krass 2006).

***Presence of natural barriers***

- Hosts of '*Ca.* L. asiaticus' are present in many parts of Australia (APNI 2008). Natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances exist between suitable hosts and may prevent long-range natural spread of the bacterium.
- The Australian citrus industry is spread across Australia in isolated regions with long distances often separating commercial orchards. This will aid in containment and eradication of the vector and/or pathogen if establishment occurs in an isolated citrus growing region (Barkley and Beattie 2008).

[11] In the absence of suitable psyllid vectors

- '*Candidatus* Liberibacter asiaticus' is heat-tolerant (Bové 2006) and arid regions surrounding many *Citrus* species production areas in Australia may not prove to be a natural barrier to the spread of this bacterium.
- A significant natural barrier to spread is the absence of suitable vectors of the bacterium in the PRA area. Natural spread of '*Ca*. L. asiaticus' is achieved by *D. citri* (da Graça 2008), which is not present in Australia. By itself, '*Ca*. L. species' can only move systemically within the plant (Spann *et al*. 2008) and through the dispersal of infected plant propagules.

***Potential for movement with commodities or conveyances***

- '*Candidatus* Liberibacter asiaticus' is spread to new areas by the introduction of infected plant material (Bové 2006). As visual symptoms may not be present, and in the absence of specific testing regimes, infected nursery stock could easily be moved to new areas.
- The introduction of infected plant material establishes the pathogen in new areas and, if unregulated movement occurs, it will accelerate the spread of the pathogen (Gottwald *et al*. 2007).
- Graft transmission has been demonstrated for '*Ca*. L. asiaticus' but not at high rates due to necrosis in sieve tubes and uneven distribution of the bacterium (Brlansky and Rogers 2007; Gottwald *et al*. 2008; Futch *et al*. 2008). Graft transmission is also variable depending on the plant part used for grafting and the amount of tissue used (Halbert and Manjunath 2004). Successful grafts can result in severely infected trees (Gonzales and Vinas 1981).
  - After grafting, symptoms may develop after eight weeks in summer and 10 weeks at other times of the year, although some test seedlings took two years or more to develop symptoms (Capoor *et al*. 1974).
  - Studies indicate that seven months after grafting diseased buds on healthy rootstock 58% of grafts had survived and of those, 20% showed disease symptoms (Lin and Lin 1990). In another experiment, 10–16% of grafts with buds from asymptomatic branches on diseased trees developed symptoms, while 40% of grafts from symptomatic branches developed symptoms in 3–9 months (Lin and Lin 1990).
  - Recent studies demonstrate a higher rate of graft transmission for '*Ca.* L. asiaticus' (Stover *et al*. 2008; Coletta-Filho *et al*. 2010). Plants grafted with infected buds sourced from symptomatic branches resulted in 100% transmission within 18 weeks (Coletta-Filho *et al*. 2010). Plants grafted with buds from infected, but asymptomatic branches, transmission rate ranged from 10 to 60% (Coletta-Filho *et al*. 2010).
  - Variation in infection rates of '*Ca*. L. asiaticus' have been observed on the plant parts used for grafting. For example, infection rates of 4.7% for stem bark, 67% for a piece of stem and 84% for a single bud has been reported (Zhao 1981).
  - Chinese box orange (*Atalantia buxifolia*) inoculated by grafting tested positive by PCR assays 2–3 months after inoculation (Hung *et al*. 2001).
- There is also potential for the spread of the bacterium through infected seed. However, research has demonstrated that while a proportion of seedlings that develop from seed from infected plants are positive for the bacterium and a proportion of these positive seedlings develop symptoms of citrus greening, the infection is transient and may be lost over time (Graham *et al*. 2008; Shatters 2008).
- Infected psyllid vectors could spread the bacterium through contaminating commodities and conveyances. *Diaphorina citri* can spread this bacterium through transport of infested host plant material including fruit (Bové 2006; Halbert and Núñez

2004; Gottwald *et al.* 2007). There are also reports from Brazil of *D. citri* being carried in empty fruit trucks (Beattie and Barkley 2009).

- Both the psyllids and the bacterium have been shown to be spread with plants for sale. Trade in *Murraya paniculata* almost undoubtedly has moved psyllid vectors carrying '*Ca*. L. asiaticus' (Halbert *et al.* 2002) and facilitated the spread of HLB throughout Florida. It is now apparent that the means and pathways of movement of *D. citri* and '*Ca*. L. asiaticus' are diverse and common, and appear to have resulted in widespread distribution of HLB in the Florida peninsula (Manjunath *et al*. 2008).
- '*Candidatus* Liberibacter asiaticus' can multiply and spread within infected *Cuscuta ceanothi*, *Cu. campestris* (Ghosh *et al*. 1977) and *Cu. australis* (Su and Huang 1990). The pathogen has been observed multiplying more favourably in *Cuscuta* spp. (dodder) than in diseased sweet oranges (Ghosh *et al*. 1977) suggesting that it could move with these host commodities. Studies indicate that infected *Cu. pentagona* did not exhibit symptoms of HLB even when they contained high titres of '*Ca.* L. asiaticus' (Zhou *et al*. 2008b). Seed sourced from infected periwinkle and *Cu. pentagona* plants resulted in the detection of the bacterium in 53% of the seeds (Zhou *et al*. 2008b).

***Potential natural enemies***

- '*Candidatus* Liberibacter asiaticus' is not known to have any natural enemies which could hamper its spread. However, certain entomophagous insects can drastically reduce psyllid populations and thus indirectly prevent an increase of the pathogen population and associated spread (Aubert 1987a).

**Spread potential if known vectors establish in Australia[12]**

- '*Candidatus* Liberibacter species' are vector specific and '*Ca.* L. asiaticus' is vectored by *Diaphorina citri* and *Trioza erytreae* (Aubert 2008).
- *Diaphorina citri* acquires the bacterium while feeding on an infected plant (Bové 2006; Lopes *et al*. 2009b). Once the psyllid has acquired the bacterium from an infected host, the psyllid maintains the bacteria and the ability to transmit the disease throughout its life (Gottwald *et al.* 2007).
- '*Candidatus* Liberibacter asiaticus' and *D. citri* are both heat tolerant (Bové 2006) and can spread in a wide range of climates. Overall, Australian climatic conditions in the commercial horticultural growing areas would support the spread of *D. citri* and associated bacteria (Barkley and Beattie 2008).
- Spread of the bacterium in the presence of the vector can be dramatic. For example, in a two year old orchard surrounded by heavily infected adult trees, the disease incidence increased from 0.6% to 27.4% in 9–10 months (Gottwald *et al*. 2007). Disease progression can also be quite fast, reaching more than 95% incidence in 3–13 years after the first symptoms occur (Gottwald *et al*. 2007). Therefore, in the presence of inoculum and the vector, the bacterium would spread quickly in most citrus growing regions of Australia.
- '*Candidatus* Liberibacter asiaticus' was first reported in 2004 in two municipalities of Brazil and, as of August 2008, had spread to 241 municipalities of São Paulo, Minas Gerais and Paraná states (Lopes 2009). In the presence of the vector the rate of spread has been estimated to be 12 miles (19 km) per year (Gottwald *et al.* 2007). Similarly, '*Ca*. L. asiaticus' was first reported in 2005 in the US state of Florida and in the presence of the psyllid has since spread to 32 counties (Halbert *et al.* 2008).

---

[12] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca.* L. asiaticus' in the presence of known vectors.

'*Candidatus* Liberibacter asiaticus' spread approximately 540 km in 10 years after the initial discovery of psyllids in the state (Halbert *et al*. 2008).

- Natural spread of the '*Ca*. L. asiaticus' is dependent on the transmission of the bacterium by its psyllid vectors (Rogers *et al.* 2008) and is related to high vector populations, percentage of psyllids carrying the bacterium and the dispersal of the psyllids (Su 2008). Generally, '*Ca*. L. asiaticus' is spread in a pattern of gradual dispersal from the primarily infected tree to the surrounding healthy trees in latent infection. Symptomless trees developed symptoms over a one year incubation period (Su 2008).
- '*Candidatus* Liberibacter asiaticus' multiplies efficiently in plants and its titres are high (Lopes *et al.* 2009b). Higher titres of this bacterium in the host plants increase the chances of pathogen acquisition and transmission by the insect vector (Lopes *et al.* 2009a).
- The extent to which '*Ca*. L. asiaticus' affects a tree depends on the age of the tree at the time of infection and the population of insect vectors. The pathogen can spread rapidly within old trees, and appears to be dependant on the infection of healthy branches through multiple feeding vectors, and not on internal movement of the bacterium (Beattie and Barkley 2009).
- Spread of the '*Ca*. L. asiaticus' by psyllid vectors will depend on the acquisition period, latency of the pathogen in the psyllid prior to transmission and transmission efficiency (Rogers *et al* 2008).
- *Diaphorina citri* can acquire '*Ca.* L. species' after 30 minutes of feeding with a latent period of 1–25 days (Halbert and Manjunath 2004).
- Adults and fourth and fifth instar life stages of *D. citri* are able to transmit '*Ca.* L. asiaticus' (Halbert and Manjunath 2004). '*Candidatus* Liberibacter asiaticus' has also been detected in third instars (Hung *et al*. 2004).
- Psyllids that complete their development on HLB infected citrus trees are more likely to acquire (and potentially transmit) '*Ca.* L. asiaticus' than psyllids that feed on HLB infected trees as adults only (Roger *et al*. 2008).
  - '*Candidatus* Liberibacter asiaticus' was detectable in 20–30% of psyllids that fed on plants as adults only, whereas up to 100% of psyllid adults that developed from nymphs on infected plant material tested positive for the presence of the HLB pathogen (Roger *et al*. 2008).
  - Adults that developed from infected nymphs transmitted the pathogen to healthy seedlings without the need for an acquisition feeding (Xu *et al*. 1988a).
- Differences in acquisition and transmission of '*Ca*. L. asiaticus' within populations of *D. citri* has also been reported (Su 2008). The Taiwanese biotype of *D. citri* is a less effective vector. Less than 5% of adults acquired the HLB pathogen after acquisition feeding on diseased citrus plant over one day, while the nymphs acquired the pathogen at a much higher rate than adults (Su 2008).
- There are vast differences reported in the literature regarding seasonal transmission of '*Ca.* L. asiaticus'. For example, peak transmission occurred from mid-spring to early-summer in Taiwan (Huang *et al*. 1990), whereas peak transmission occurred from early- to mid-spring in India (Chavan 2004). Transmission to pathogen-free sweet orange seedlings by nymphs collected from HLB infected trees in early spring was 67%, compared to 33% in summer; transmission by adults was 45–50% in early- to mid-spring.
- Seasonal levels of '*Ca.* L. asiaticus' infection in psyllid populations has been studied in Florida (Manjunath *et al*. 2008). The highest levels of infections were found in autumn,

probably coinciding with feeding on autumn flushes. The results suggested that, even though psyllid populations may reach a peak during spring flush, higher incidences of '*Ca*. L. asiaticus' infection may occur in autumn when populations are much lower. The populations rose rapidly from low levels in winter to peak in spring, before declining to low levels again by late summer.

- Bové *et al*. (1993) also reported seasonal fluctuations in the number of infected psyllids; with more occurring among adults in autumn (39%) and few (less than 1%) in winter. '*Candidatus* Liberibacter asiaticus' incidence among *D. citri* populations collected on HLB affected citrus plants in the field in Florida varied from 10–100% (Li *et al*. 2008).
- The presence of natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances between suitable hosts, may limit the ability of *D. citri* to spread '*Ca*. L. asiaticus' to new areas.
- *Diaphorina citri* is capable of moving independently at least 8–60 m (Hall 2008a) and adults have been estimated to be transported in wind drifts for 0.5–1 km (Barkley and Beattie 2008). Furthermore, there is some evidence for occasional adult mass migrations (Hall 2008a).
- Bass Strait may act as a natural barrier to *D. citri* carrying '*Ca.* L. asiaticus' from mainland Australia to Tasmania as the psyllids would not be capable of dispersing the distance over the strait. However, *D. citri* also have the potential to move large distances (over 90–470 km) on seasonal trade winds or hurricanes (Bové 2006; Sakamaki 2005; Tsai 2006; Gottwald *et al*. 2007; Halbert *et al*. 2008).
  - Field studies in the Philippines and China suggest possible medium to long distance transport of *D. citri* by strong winds in open orchards without windbreak protection (Aubert 1987a).
  - Sakamaki (2005) speculated that migration of the psyllid in the Okinawa Islands in southeast Japan is governed by seasonal winds. It possible for the psyllid to migrate 470 km northwards to the large island of Kyushu over sea by riding lower jet airstreams associated with summer monsoons.
  - Gottwald *et al*. (2007) also speculated that *D. citri* may have been carried some 90–145 km by wind over non-citrus growing regions in Florida. This long-distance movement may have been related to air masses during hurricanes or tropical storms.
  - There is circumstantial evidence that infected *D. citri* flew across the Florida Everglades and infected the eastern borders of large commercial citrus groves just to the west of the Everglades (Manjunath *et al*. 2008).
  - '*Candidatus* Liberibacter asiaticus' has recently been discovered in Cuba. da Graça (2008) speculated that infected psyllids from Florida crossed the short distance to Cuba, and infected trees there.
- Passive movement of the psyllid indicates that infected psyllids could overcome the natural barriers in Australia and spread the bacterium in suitable environments.
- *Diaphorina citri* feeds and reproduces on the Rutaceae, including cultivated *Citrus* species and Australian naturalised/native species, *Murraya paniculata* and *Citrus australasica* (Halbert and Manjunath 2004). These hosts are widespread in the PRA area. Most, but not all, host species of *D. citri* are also hosts of '*Ca*. L. asiaticus'. This makes it increasingly likely that the vector would spread the bacterium to suitable hosts.

- *D. citri* adults infected with '*Ca.* L. asiaticus' have been proven to be capable of transmitting the bacterium to *Murraya paniculata*, which can then act as a reservoir for up to 18 months. *D. citri* can also acquire '*Ca.* L. asiaticus' and infect *Citrus* species (Damsteegt *et al.* 2010).
- Suitable hosts of *D. citri* and the bacterium are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).
- Once '*Ca.* L. species' are detected in a citrus orchard, in the absence of control measures, known vectors have been recorded to spread the pathogen to 100% of trees in five years (Bové 2006).

The suitability of the environment, the presence of multiple host species throughout the PRA area, the potential to disperse in propagative material and the potential for rapid spread with vectors supports an assessment of 'high' for the spread of '*Ca* L. asiaticus' in the presence of known vectors.

**Spread potential in the absence of known vector[13]**

- In the absence of the vectors, the most likely means of spread of '*Ca.* L. asiaticus' would be through the movement of infected planting material (Polek *et al.* 2007). If infected budwood is used for grafting it will help spread the bacterium to non-infested areas.
- If '*Ca*. L. asiaticus' was to establish in Australia it would likely lead to the death of its infected host plants and not pose a severe threat to the citrus industry (Barkley and Beattie 2008).
- Although one species of *Diaphorina* (*D. tryoni*) and five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) are present in Australia none on them feed on the Rutaceae (DEWHA 2009). Therefore these species are unlikely to act as vectors.
- Species of psyllids other than *D. citri* and *T. erytreae* have been recorded on Rutaceous hosts, where this bacterium is present, but none have been shown to be vectors (Gottwald *et al*. 2007). Therefore, the psyllids *Anomalopsylla* sp. n., *Ctenarytaina thysanura* and *Geijerolyma robusta*, known to feed on Rutaceous hosts in Australia (DEWHA 2009), are highly unlikely to act as vectors.

In the absence of *D. citri* from Australia, the assessment supports a rating of 'very low' for the spread of '*Ca* L. asiaticus'.

### 5.3.4 Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probability of entry, of establishment and of spread using the matrix of rules for combining descriptive likelihood (Table 2.2).

- The overall likelihood that '*Ca.* L. asiaticus' will enter Australia by the pathways discussed in this PRA, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia are set out in Table 5.8.

---

[13] The information presented under this heading is only applicable to the assessment of probability of spread of '*Ca.* L. asiaticus' in the absence of known vectors.

**Table 5.8: Overall probability of entry, establishment and spread of '*Ca.* L. asiaticus' by different pathways**

| Pathway | Probability of | | | Overall probability of entry, establishment and spread |
|---|---|---|---|---|
| | Entry | Establishment | Spread | |
| Fruit (containing seed) | Extremely low | High | High (Very low)* | Extremely low (Extremely low) |
| Nursery stock (live plants) | High | | | High (Very low) |
| Budwood | Moderate | | | Moderate (Very low) |
| Fresh leaves | Extremely low | | | Extremely low (Extremely low) |
| Seed for sowing | Low | | | Low (Very low) |
| Infected psyllids | Moderate | | | Moderate |

* Ratings in parenthesis are in the absence of known vectors in Australia

### 5.3.5 Consequences

The consequences of the entry, establishment and spread of '*Ca.* L. asiaticus' in Australia have been estimated according to the methods described in Table 2.3. The assessment of potential consequences for the bacterium is treated in combination with its psyllid vector as HLB has never been detected in the absence of a vector and economic consequences have never been assessed in isolation. The assessment is provided below:

| **Criterion** | **Estimate and rationale** |
|---|---|
| **Direct** | |
| **Plant life or health** | **Impact score**: **E** – Significant at the regional level.<br>• '*Ca.* L. asiaticus' is a highly destructive pathogen and is the major limiting factor for citrus production in parts of Asia (Bové 2006; Gottwald *et al*. 2007) and its impact in the Americas is increasing as the pathogen, and its vector, spread through the region (Gottwald *et al*. 2007).<br>• '*Candidatus* Liberibacter asiaticus' infects both plants and fruit (Bové 2006). Infected leaves generally become hard and curl outwards after getting old and occasionally have veined corking. This is followed by premature defoliation, dieback of twigs, decay of feeder and lateral roots, decline in vigour and ultimately death of the entire plant (da Graça 1991).<br>• Trees infected at an early age may fail to produce fruit while more mature trees become unproductive soon after infection. It is the primary cause of losses in citrus production in Asia (Aubert 1990) and there is no successful treatment of infected trees.<br>• Excessive fruit drop occurs, and remaining fruit are often lop-sided, remain green at the stylar end, contain aborted seed and discoloured vascular bundles, and can have a bitter taste (da Graça 2008). Fruit produced by infected trees has an unpalatable flavour (Husain and Nath 1927; da Graça 1991) and may taint extracted juice products with consequent economic loss.<br>• '*Candidatus* Liberibacter asiaticus' has spread widely and caused great damage to the citrus industry by shortening tree lifespan to less than 10 years and lowering fruit quality in recent decades in tropical and subtropical Asian and Pacific regions (Su 2008).<br>• In areas where HLB is established and the vector is present, the damage to citrus production can be extremely high.<br>  – Decline and loss of productivity of citrus trees in the Philippines due to HLB began in 1957 (Martinez and Wallace 1967). In Batanges Province, citrus production rose between 1955 and 1960 to a peak of 11,680 t before declining to 6,300 t in 1964 and 1,000 t in 1968 following the detection and spread of HLB. Sixty to ninety percent of trees were infected in many orchards, causing their abandonment (Martinez and Wallace 1968; Martinez *et al.* 1971), and more than one million trees were reportedly in decline (Martinez and Wallace 1967).<br>  – Huanglongbing destroys 10–15% of tangerine trees in Thailand annually (Bové 2006).<br>  – In Réunion, an eight year survey conducted in the 1980s and 1990s determined that 65% of trees were infected and became unproductive within seven years of planting (Gottwald *et al.* 1989; Gottwald *et al.* 2007).<br>• Some Australian Rutaceae have been tested for susceptibility to '*Ca*. L. species' (Halbert and Manjunath 2004). Several native and naturalised species demonstrate disease symptoms following natural infection and many more species are suspected as being hosts, but have not undergone susceptibility trials.<br>• None of the native Australian Rutaceae considered potential hosts of '*Ca.* L. species' (Beattie and Barkley 2009) are rare or endangered (DEWHA 2009).<br>• Some hosts of '*Ca*. L. asiaticus' constitute a component of garden environments. Such hosts are sporadically distributed and any impact would be restricted to the local levels. |
| **Other aspects of the environment** | **Impact score**: **B** – Minor significance at the local level.<br>There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>• In general, newly established species may affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants degrade critical habitat or stimulate use of chemicals or biological controls. There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>  – '*Candidatus* Liberibacter asiaticus' is unlikely to affect the environment in these ways, as the bacterium infects and multiply only in the Rutaceae. |
| **Indirect** | |

| Criterion | Estimate and rationale |
|---|---|
| **Eradication, control etc.** | **Impact score**: **F** – Significant at the national level.<br>• Huanglongbing symptoms abate only temporarily when trees are injected with antibiotics; the disease is not eliminated by antibiotic treatment (Halbert and Manjunath 2004). Consequently prevention is the only reliable control method.<br>• Once established, '*Ca*. L. asiaticus' is both difficult and expensive to eradicate. Eradication of the bacterium would require the removal of large numbers of native, amenity and commercial citrus plants within the vicinity of outbreaks. Due to the large number of host plants affected, the costs of any eradication campaign are likely to be substantial and spread across a number of landholders.<br>• Citrus rehabilitation was attempted in northern Bali, Indonesia, where the incidence of HLB was high, by total citrus eradication in all orchards and backyards, then planting up to one million mandarin trees propagated from HLB free buds. But replanting commenced before eradication was complete and within 10 years almost 100% of newly planted trees were affected (Bové *et al.* 2000).<br>• General control methods for '*Ca*. L. asiaticus' include the use of certified greening-free nursery trees, systemic insecticides to control the psyllid vector, and removal of diseased trees to reduce inoculum levels. Implementing these measures would require a multifaceted and costly approach.<br>• While potentially able to be managed in commercial production, the presence of the bacterium will significantly increase the production costs for producers. For example, in Florida it is estimated that production costs associated with an aggressive management program for the *D. citri* and HLB are 40% greater than the pre-HLB costs (Irey *et al.* 2008).<br>• Control of psyllid vectors is the key to limiting the impact of pathogen. Foliar sprays (imidacloprid) have been used to control the psyllid vectors of the disease (Grafton-Cardwell *et al*. 2006).<br>• Considering the extreme fertility of the psyllid vector, with each female laying as many as 1000 to 2000 eggs in a matter of three weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment (Aubert 2008). |
| **Domestic trade** | **Impact score**: **D** – Significant at district level<br>• '*Candidatus* Liberibacter asiaticus' now occurs throughout the state of Florida and threatens a citrus industry valued at $1.3 billion (US) in direct sales alone (Qureshi and Stansly 2010).<br>• The presence of '*Ca*. L. asiaticus' in production areas may result in some domestic movement restriction for host commodities. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock and seed for sowing.<br>• Interstate restrictions on nursery stock of the Rutaceae may lead to a loss of markets, which in turn would be likely to require industry adjustment. |
| **International trade** | **Impact score**: **D** – Significant at district level<br>• The presence of '*Ca*. L. asiaticus' in production areas may limit access to some overseas markets where the pathogen is absent. However, due to the extremely low risk of entry of the pathogen posed by movement of fruit for consumption, restrictions are only likely for nursery stock and seed for sowing. |
| **Environmental and non-commercial** | **Impact score**: **C** – Significant at the local level.<br>• Large scale removal of native hosts and infected trees would have significant effects on the environment. Broad-scale chemical treatments directed against known insect vectors may also have some impacts on native insects.<br>• Historically, the introduction of invasive agricultural pests has initiated control measures to avoid lost production. Consumer preferences for unblemished, high quality produce encourage the use of pesticides, while at the same time, negative public opinion regarding the use of pesticides on fruits and vegetables is a market concern (Bunn *et al*. 1990). Therefore, the establishment of any new pests of fruits and vegetables destined for fresh markets is likely to stimulate greater use of either chemical or biological controls to ensure continued market access and market share.<br>• Twenty insecticide applications per year would be required to control the psyllid vector (Lopes *et al.* 2008). Wind dispersal of dry orchard soil containing broad spectrum chemicals may also have other impacts on the local environment. |

Based on the decision rules described in Table 2.4, where the consequences of a pest with respect to one or more criteria are '**F**', the overall consequences are considered to be **HIGH**.

### 5.3.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for '*Ca*. L. asiaticus' is summarised in Table 5.9.

**Table 5.9: Unrestricted risk estimates of '*Ca*. L. asiaticus' for different pathways**

| Pathway | Overall probability of entry, establishment and spread | Consequences | Unrestricted risk |
|---|---|---|---|
| Fruit (containing seed) | Extremely low (Extremely low) | High | Very low (Very low) |
| Nursery stock (live plants) | High (Very low) | | High (Low) |
| Budwood | Moderate (Very low) | | High (Low) |
| Fresh leaves | Extremely low (Extremely low) | | Very low (Very low) |
| Seed for sowing | Low (Very low) | | Moderate (Low) |
| Infected psyllids | Moderate | | High |

* Ratings in parenthesis are in the absence of known vectors in Australia

The unrestricted risk for '*Ca*. L. asiaticus' has been assessed as 'high–low' for nursery stock and budwood and 'high' for psyllid vectors, and 'moderate–low' for seed for sowing, which are all above Australia's ALOP. Therefore, specific risk management measures are required for '*Ca*. L. asiaticus' for nursery stock, budwood, seed for sowing and psyllid vectors. The unrestricted risk for '*Ca*. L. asiaticus' has been assessed as 'very low' for fruit and fresh leaves which is below Australia's ALOP. Therefore, no management measures for these pathways are required.

## 5.4 *Diaphorina citri*

*Diaphorina citri* evolved in India in association with a species of *Murraya* (Hollis 1987; Halbert and Manjunath 2004), but was first described from *Citrus* species in Taiwan in 1907 (Halbert and Manjunath 2004). *Diaphorina citri* damages plants directly through its feeding activities on *Citrus* species and closely related ornamentals (Halbert and Manjunath 2004). New shoot growth that is heavily infested by the psyllid does not expand and develop normally, and is more susceptible to breaking-off (Grafton-Cardwell *et al.* 2006). Psyllids extract large quantities of sap from the plant as they feed and produce copious amounts of honeydew. The honeydew coats the leaves of the tree, encouraging sooty mould to grow that can reduce photosynthetic activity (Grafton-Cardwell *et al.* 2006).

*Diaphorina citri* is the natural vector of '*Candidatus* Liberibacter asiaticus' and '*Ca.* L. americanus', which cause Huanglongbing (Bové 2006). In Réunion, *D. citri* is also a natural vector of '*Ca.* L. africanus' (Aubert 2008). Within *D. citri* populations some biotype differences (acquisition and transmission of the bacterium) are discernible (Su 2008). The Taiwanese biotype of *D. citri* is less efficient at HLB transmission. Less than 5% of adults acquired the HLB pathogen after acquisition feeding on diseased citrus plants over one day, while the nymphs acquired the pathogen at much higher rates than adults (Su 2008).

### 5.4.1 Probability of entry

### Pathway 1—Fruit

#### Probability of importation

The likelihood that *Diaphorina citri* will arrive in Australia with trade in fruit from countries where the insect is present is **MODERATE**.

***Association of the pest with the pathway***

- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004; Aubert 1987b). All life stages of *D. citri* are found on host foliage throughout its range (Gottwald *et al.* 2007).
- There are no records of *D. citri* laying eggs on fruits, nymphs completing their development on fruit, or nymphs and adults feeding on mature fruit (Gottwald *et al.* 2007). However, nymphs can feed on newly formed fruits of host plants (Brlansky and Rogers 2007).
- *Diaphorina citri* completes its nymphal development in 11–15 days (Halbert and Manjunath 2004). Citrus fruit maturation ranges from 6–7 months in the low tropics to 14–16 months in Mediterranean-type climates (Davies and Albrigo 1994). Therefore, if nymphs feeding on immature fruit could complete their development to the adult stage they are likely to do so prior to fruit harvest.
- Although mature fruit are not attractive feeding sites for psyllids, foliage is an attractive site for oviposition, nymphal development, adult feeding and overwintering (Bové 2006; Yang *et al*. 2006; Pluke *et al.* 2008).
- The overwintering period for adults is likely to coincide with the harvest times for citrus fruits. The presence of large numbers of adults on the foliage of citrus trees in winter increases the likelihood of the psyllid being associated with fruit at harvest as hitchhikers.
- Consignments of citrus fruit with leaf material as a contaminant also presents a risk of entry of *D. citri*. Leaf material contaminating fruit may carry eggs or immature stages of the psyllid as eggs are laid on leaves and immature stages feeding on leaves are sessile (Aubert 1987b; Grafton-Cardwell *et al*. 2006).
- Eggs and immature stages of *D. citri* are small (eggs measuring 0.31mm and early instars measuring 0.22–1.58 mm (EPPO 2005b; Mead 2008) and may escape detection during routine visual inspection.
- Adult psyllids are attracted to bright lights (Barkley and Beattie 2008) and may therefore infest packing houses. Consequently, there is a risk of psyllids contaminating the fruit in packing houses.
- Live *D. citri*, sometimes in large numbers, have been intercepted on unprocessed citrus fruit from the Bahamas shipped to Florida for processing (Halbert and Núñez 2004; Sullivan and Zink 2007).
- *Diaphorina citri* has been intercepted from Belize on citrus in baggage at Houston Airport, Texas (Halbert and Núñez 2004). The psyllid has also been detected on citrus fruit from Mexico into Texas (Halbert and Núñez 2004).

***Ability of the pest to survive transport and storage***

- Fruit destined for Australia would be shipped in refrigerated containers maintained at 4–6 ºC. These storage conditions are unlikely to have a significant impact on *D. citri* in imported fruit.

- *Diaphorina citri* is resilient to temperature (Hall 2008b; Barkley and Beattie 2008) and is known to survive, without food or water, for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001).
- Adult *D. citri* are able to survive 52.5–94.5 hours at 25 °C without feeding if suitable foliage is not available (McFarland and Hoy 2001). Therefore, psyllids present on fruit could survive for longer periods during transportation and storage under low temperatures.
- The interception of live *D. citri* on unprocessed citrus fruit (Halbert and Núñez 2004; Sullivan and Zink 2007) indicates that this species is able to survive transportation and storage.

***Ability of the pest to survive existing pest management procedures***

- Chemicals are used to control the psyllid in citrus orchards in Réunion and mainland China (Aubert and Quilici 1984; Xu *et al*. 1991). Neem oil and petroleum spray oil have been trialled for use in India and China (Chakravarthi *et al*. 1998; Rae *et al*. 1997).
- The parasite, *Tamarixia radiata* has been used to control *D. citri* (Aubert *et al*. 1980). Other members of Syrphidae and Coccinellidae have been reported to feed on *D. citri*. These control methods are likely to reduce but not necessarily eliminate *D. citri*.

None of the life stages of this psyllid are directly associated with mature fruit; however, they may be associated indirectly as adult hitchhikers, or as eggs or nymphs on trash contaminating consignments. The psyllid has also been intercepted on unprocessed fruit. Therefore, an assessment of 'moderate' for the importation of this psyllid in fruit is allocated.

## Probability of distribution

The likelihood that *Diaphorina citri* will be distributed within Australia with fruit sourced from countries where the pest is present and be able to transfer to suitable hosts is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- Adult *D. citri* associated with imported fruit are able to move independently from the pathway to a suitable host. Although they are considered weak fliers, adults have been recorded to regularly fly distances of 8–60 m (Hall 2008a) and are transported 0.5–1 km in wind drifts (Barkley and Beattie 2008), and are able to survive up to 52.5–94.5 hours without feeding if suitable foliage is not available (McFarland and Hoy 2001).
- Nymphs are able to crawl short distances to suitable hosts if brought into close contact with another host plant (Barkley and Beattie 2008).
- Eggs or nymphs arriving in Australia on trash associated with imported fruit would still need to complete their development into adults. The life cycle of this species varies from 15–47 days from egg to adult, and the adults survive for several months thereafter, depending on climatic conditions (Mead 2008).
- However, feeding of nymphs is generally restricted to the young tender leaves on which eggs were laid and occasionally young fruits (Brlansky and Rogers 2007). There are no records of *D. citri* completing development from egg to adult on trash or mature fruit.
- The host range of Asian citrus psyllid includes 25 genera in the family Rutaceae, although not all of these are good hosts (Halbert and Manjunath 2004). Their preferred hosts are in the genera *Citropsis*, *Citrus* and *Murraya* (Grafton-Cardwell *et al.* 2006).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

***Distribution of the imported commodity in the PRA area***

- Fruit would be distributed to multiple destinations throughout Australia for retail sale. Therefore, any adults or immature life stages associated with fruit would be distributed to multiple locations.
- *Diaphorina citri* would need to survive transportation and storage within the PRA area. *Diaphorina citri* survives in a wide range of temperatures extremes, from 45 °C in Saudi Arabia to -7 °C or -8 °C in subtropical China (Aubert 1988, 1990) and is therefore likely to survive a range of transport and storage conditions.
- Thus, *D. citri* present on fruit distributed for retail or market sale, have the ability to survive transport and storage conditions and be distributed throughout the PRA area.

***Risks from by-products and waste***

- Although the intended use of fresh fruit is human consumption, waste material would be generated (e.g. overripe and damaged fruit, uneaten portions and trash). Whole or parts of the fruit may be disposed of at multiple locations throughout Australia in compost bins or amongst general household or retail waste.
- *Diaphorina citri* may survive on waste material for a short time before dispersing to suitable hosts. However, *D. citri* feed preferably on tender leaves and the tender portions of plant branches and succulent stems (Brlansky and Rogers 2007) and are unlikely to feed or survive for long on discarded waste.
- *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to move independently (Hall 2008a).
- Adult *D. citri* are also known to survive, without food or water for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001). For example, at 25 °C, 50% mortality was observed at 7%, 33%, 53%, 73% and 97% relative humidity for 24.9 hours, 28.3 hours, 28.5 hours, 37 hours and 43.2 hours, respectively (McFarland and Hoy 2001).

The ability for psyllids to disperse both independently and through the movement of infested fruits, and the presence of numerous host species in the PRA area supports an assessment of 'high' for this species.

## Pathway 2—Nursery stock (live plants)

### Probability of importation

The likelihood that *Diaphorina citri* will arrive in Australia with trade in nursery stock (live plants) from countries where the insect is present is **HIGH**.

***Association of the pest with the pathway***

- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004), so nursery stock of host plants can be infested and provide a pathway for the importation of the psyllid into Australia.
- Adults of *D. citri* are present throughout the year and populations are attracted to the volatiles emitted from new shoot flushes of Rutaceous host plants (Patt and Sétamou 2010).
- High psyllid population densities are often found in citrus nurseries, since the young trees are maintained in a state of almost constant growth (Floyd and Krass 2006). Active growth on alternate plant hosts support psyllid populations when citrus flush is not available (Floyd and Krass 2006).
- Eggs are laid on the tips of growing shoots or in the crevices of unfolded 'feather flush' leaves (Grafton-Cardwell *et al*. 2006). *Diaphorina citri* eggs are small (0.31 mm in

length), bright yellow-orange and would be difficult to detect during routine visual inspection (Aubert 1987b; EPPO 2005b).

- *Diaphorina citri* nymphs are green or dull orange, and are sedentary on leaves, the terminal stem and between the axillary bud and the stem of tender shoots (Aubert 1987b; CABI 2007). Nymphs are flat and tend to wrap themselves around the shoot where they feed, which may make visual detection difficult (Halbert and Manjunath 2004). First–fifth instar stages measure 0.25–0.35 mm, 0.49–0.53 mm, 0.69–0.72 mm, 0.98–1.05 mm and 1.45–1.58 mm in length, respectively (EPPO 2005b; Mead 2008). These life stages would be difficult to detect during routine visual inspection.
- Adults of *D. citri* are 2.5 mm in length (EPPO 2009), and may be detected with the naked eye.
- Therefore, live plants from infected areas may carry adults, eggs and/or nymphs over longer distances (Grafton-Cardwell *et al*. 2006; Tsai 2006).
- *Diaphorina citri* has been introduced through retail trade in *Murraya paniculata* plants throughout Florida and Texas in the USA (CDFA 2008a), indicating that nursery stock is a primary pathway for the introduction of this psyllid.
- There have been 170 interceptions of live *D. citri* at ports in the USA, on plant material with an Asian origin, from 1985–2003 (Halbert and Manjunath 2004; Sullivan and Zink 2007). There are an additional 73 records of interceptions of live *Diaphorina* species on Rutaceous plants from Asia (Halbert and Manjunath 2004).

***Ability of the pest to survive transport and storage***

- *Diaphorina citri* is able to survive transport and storage as the psyllid is resilient to temperature (Hall 2008b; Barkley and Beattie 2008). Nursery stock is expected to be shipped at moderate temperatures and humidity levels which are unlikely to adversely affect any *D. citri* populations that are present. The transport temperature for citrus plant material is typically cool to cold and is unlikely to affect psyllid survival and may in fact prolong their life.
- *Diaphorina citri* has been introduced into new areas through the importation of infested planting material (CDFA 2008a). The interception of live *D. citri* on *Murraya* and *Citrus* species in the USA (Halbert and Manjunath 2004; Sullivan and Zink 2007) indicates that this species is able to survive transportation and storage.
- Nursery stock can carry eggs and nymphs of *Diaphorina citri*. The nymphal stages associated with imported nursery stock are fairly sedentary and would need to complete their development into adults on their current host. In the case of nursery stock, this species has the potential to develop from eggs to nymphs during transport and storage as the host will provide ample food supply during transit.
- Humidity, temperature and host plant effects will play a role in the survival and developmental rate of this psyllid. Optimal adult survival is at humidity above 53%, but there is significant survival at humidity as low as 7% (McFarland and Hoy 2001).

***Ability of the pest to survive existing pest management procedures***

- Chemicals are used to control the psyllid in citrus orchards in Réunion and mainland China (Aubert and Quilici 1984; Xu *et al*. 1991). Neem oil and petroleum spray oil have been trialled for use in India and China (Chakravarthi *et al*. 1998; Rae *et al*. 1997).
- The parasite, *Tamarixia radiata* has been used to control *D. citri* (Aubert *et al*. 1980). Other members of Syrphidae and Coccinellidae have been reported to feed on *D. citri*. These control methods will likely reduce, but not necessarily eliminate, *D. citri*.

The small size of the pest, association of all life stages with the commodity, likelihood of the species remaining active during transport and storage, and numerous interception records support an assessment of 'high' for the importation of this species with nursery stock.

## Probability of distribution

The likelihood that *Diaphorina citri* will be distributed within Australia in a viable state with imported nursery stock (live plants) from countries where the insect is present is **HIGH**.

#### *Ability of the pest to move from the pathway to a suitable host*

- *Diaphorina citri* arriving in Australia with nursery stock will not need to move from the import pathway to a suitable host as the psyllid is able to develop, reproduce and complete its life cycle without leaving the host.
- Natural movement of *D. citri* from infested nursery stock to a suitable host will be by active flight and by passive transportation via wind currents (Hall 2008a). Although they are considered weak fliers, adults of *D. citri* have been recorded to regularly fly distances of 8–60 m (Hall 2008a), and are transported 0.5–1 km in wind drifts (Barkley and Beattie 2008).
- Nymphs are able to crawl short distances to suitable hosts if brought into close contact with another host plant (Barkley and Beattie 2008).
- Eggs or nymphs arriving in Australia on nursery stock would still need to complete their development into adults. The life cycle of this species varies from 15–47 days from egg to adult, and the adults survive for several months thereafter, depending on climatic conditions (Mead 2008).
- The host range of Asian citrus psyllid includes 25 genera in the family Rutaceae, although not all of these are good hosts (Halbert and Manjunath 2004). Their preferred hosts are in the genera *Citropsis*, *Citrus* and *Murraya* (Grafton-Cardwell *et al.* 2006).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

#### *Distribution of the imported commodity in the PRA area*

- The distribution of nursery stock would be for wholesale or retail distribution to multiple destinations throughout Australia. Nursery stock would be distributed for commercial or amenity growing purposes.
- *Diaphorina citri* would need to survive transportation and storage within the PRA area. Nursery stock is expected to be maintained at moderate temperatures and humidity levels to ensure nursery stock survival, so a portion of infested propagation material that enters the country is likely to reach areas of host abundance.
- In the case of plant material for commercial use, if infested nursery stock was introduced, the psyllids could potentially move to other suitable host plants surrounding them, and be passively redistributed by the further movement of these infested plants.
- As nursery stock would be distributed throughout Australia and planted directly into suitable habitats there is no need for *D. citri* to be transported to a suitable host.

#### *Risks from by-products and waste*

- Although the intended use of live plants is propagation, waste material would be generated. Whole or parts of the plants may be disposed of at multiple locations throughout Australia in compost bins or amongst general household waste or green waste.

- Any immature life stage of the psyllid associated with waste material would still need to complete their development into adults. However, *D. citri* feed preferably on tender leaves and the tender portions of plant branches and succulent stems (Brlansky and Rogers 2007) and are unlikely to feed or survive for long on discarded waste.
- *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to move independently (Hall 2008a).
- Adult *D. citri* are known to survive, without food or water for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001). For example, at 25 °C, 50% mortality was observed at 7%, 33%, 53%, 73% and 97% relative humidity for 24.9 hours, 28.3 hours, 28.5 hours, 37 hours and 43.2 hours, respectively (McFarland and Hoy 2001).

The association of all life stages with the commodity, the ability of psyllids to disperse both independently and through the movement of infested nursery stock, and the presence of numerous host species supports an assessment of 'high' for the distribution of this psyllid in nursery stock.

## Pathway 3—Budwood

### Probability of importation

The likelihood that *Diaphorina citri* will arrive in Australia with trade in budwood from countries where the insect is present is **LOW**.

#### *Association of the pest with the pathway*

- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004). Nymphs and adults of *D. citri* are known to feed on stems and twigs of *Citrus* species (Halbert and Manjunath 2004; Bové 2006) and are considered to be associated with budwood (EPPO 2009). Therefore budwood could provide a pathway for the importation of the psyllid into Australia.
- *Diaphorina citri* eggs may also be associated with budwood (EPPO 2009). However, they are typically laid on the tips of growing shoots or in the crevices of unfolded 'feather flush' leaves (Grafton-Cardwell *et al*. 2006).
- Budwood is typically sourced from older (e.g. one-year-old growth), foliage-free stems, with dormant buds suitable for grafting. Therefore, budwood is less likely to harbour eggs and feeding nymphs or adults as *D. citri* feed preferably on tender leaves and the tender portions of plant branches and succulent stems (Brlansky and Rogers 2007).
- *Diaphorina citri* eggs (0.31 mm in length) and nymphal stages (0.22–0.35 mm, 0.49–0.53 mm, 0.69–0.72 mm, 0.98–1.05mm and 1.45–1.58 mm in length) are small (EPPO 2005b; Mead 2008) and would be difficult to detect during routine visual inspection.
- Adults of *D. citri* are 2.5 mm in length (EPPO 2009), and may be detected with the naked eye.

#### *Ability of the pest to survive transport and storage*

- *Diaphorina citri* is able to survive transport and storage as the insect is resilient to temperatures (Hall 2008b; Barkley and Beattie 2008). Budwood is expected to be shipped at moderate temperatures and humidity levels which are unlikely to adversely affect any *D. citri* populations that are present. The transport temperature for citrus plant material is typically cool to cold and is unlikely to affect adult psyllid survival and may in fact prolong their life.
- As budwood is not the preferred feeding site for nymphs, as it is typically free from new growth, the survival of nymphs on budwood may be lower than on new plant

growth. However, adult *D. citri* are able to survive up to 52.5–94.5 hours without feeding if suitable material is not available (McFarland and Hoy 2001).
- Adult *D. citri* are also known to survive, without food or water for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001). For example, at 25 °C, 50% mortality was observed at 7%, 33%, 53%, 73% and 97% relative humidity for 24.9 hours, 28.3 hours, 28.5 hours, 37 hours and 43.2 hours, respectively (McFarland and Hoy 2001). As budwood is likely to be stored at lower temperatures than cited in McFarland and Hoy (2001) it is likely *D. citri* can survive for longer the periods of time at lower temperatures.

***Ability of the pest to survive existing pest management procedures***
- Chemicals are used to control the psyllid in citrus orchards in Réunion and mainland China (Aubert and Quilici 1984; Xu *et al*. 1991). Neem oil and petroleum spray oil have been trialled for use in India and China (Chakravarthi *et al*. 1998; Rae *et al*. 1997).
- The parasite, *Tamarixia radiata* has been used to control *D. citri* (Aubert *et al*. 1980). Other members of Syrphidae and Coccinellidae have been reported to feed on *D. citri*. These control methods will likely reduce, but not necessarily eliminate, *D. citri*.

Although the preferred feeding sites for psyllids are new leaf and shoot growth, they may be associated with budwood at low levels and are likely to survive transport and storage. Therefore an assessment of 'low' for the importation of this species with budwood is given.

## Probability of distribution

The likelihood that *Diaphorina citri* will be distributed within Australia in a viable state with imported budwood from countries where the insect is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***
- Adult *D. citri* associated with imported budwood are able to move independently from the pathway to a suitable host. Although they are considered weak fliers, adults have been recorded to regularly fly distances of 8–60 m (Hall 2008a) and are transported 0.5–1 km in wind drifts (Barkley and Beattie 2008), and are able to survive up to 52.5–94.5 without feeding if suitable foliage is not available (McFarland and Hoy 2001).
- Eggs or nymphs arriving in Australia on budwood would still need to complete their development into adults. The life cycle of this species varies from 15–47 days from egg to adult, and the adults survive for several months thereafter, depending on climatic conditions (Mead 2008).
- However, feeding of nymphs is generally restricted to the young tender leaves on which eggs were laid (Brlansky and Rogers 2007). Nymphs are able to crawl short distances to suitable hosts if brought into close contact with another host plant (Barkley and Beattie 2008).
- The host range of Asian citrus psyllid includes 25 genera in the family Rutaceae, although not all of these are good hosts (Halbert and Manjunath 2004). Their preferred hosts are in the genera *Citropsis*, *Citrus* and *Murraya* (Grafton-Cardwell *et al.* 2006).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

***Distribution of the imported commodity in the PRA area***
- Budwood is imported specifically for the purpose of grafting onto local rootstock and would be distributed to multiple destinations throughout Australia. Distribution of

infested budwood commercially through nurseries would facilitate the distribution of *D. citri*.
- *Diaphorina citri* would need to survive transportation and storage within the PRA area. Budwood is expected to be maintained at moderate temperatures and humidity levels to ensure budwood survival, so a portion of infested propagation material that enters the country is likely to reach areas of host abundance.
- Thus *D. citri* associated with budwood have the ability to survive transport and storage conditions and be distributed throughout the PRA area.

***Risks from by-products and waste***
- Although the intended use of budwood is for propagation, and all imported material is likely to be used, waste material may be generated. Whole or parts of the budwood may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- Any immature life stage of the psyllid associated with disposed material would still need to complete their development into adults. However, *D. citri* feed preferably on tender leaves and the tender portions of plant branches and succulent stems (Brlansky and Rogers 2007) and are unlikely to feed or survive for long on discarded waste.
- *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to move independently (Hall 2008a).
- Adult *D. citri* are also known to survive, without food or water for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001). For example, at 25 °C, 50% mortality was observed at 7%, 33%, 53%, 73% and 97% relative humidity for 24.9 hours, 28.3 hours, 28.5 hours, 37 hours and 43.2 hours, respectively (McFarland and Hoy 2001).

The ability of *D. citri* to disperse both independently and through the movement of infested budwood, and the presence of numerous hosts species in the PRA area support an assessment of 'high' for the distribution of this species on budwood.

## Pathway 4—Fresh leaves

### Probability of importation

The likelihood that *Diaphorina citri* will arrive in Australia with trade in fresh leaves from countries where the insect is present is **HIGH**.

***Association of the pest with the pathway***
- *Diaphorina citri* feeds and breeds on a wide range of plants within the Rutaceae (Halbert and Manjunath 2004) and its reproductive biology is closely tied to the availability of new leaf flush for egg laying and subsequent development of psyllid nymphs (Bové 2006).
- Adult females lay eggs on the tips of growing shoots or in the crevices of unfolded 'feather flush' leaves (Grafton-Cardwell *et al*. 2006). Nymphal stages of *D. citri* develop exclusively on newly expanding shoots of citrus and related species of the Rutaceae (Shivankar *et al*. 2000) and are sedentary on leaves, on the terminal stem and between the axillary bud and the stem of tender shoots (CABI 2007). Therefore, fresh leaves may harbour *D. citri* eggs, nymphs and/or adults.
- Females' prefer flush growth that is < 6 mm in length to longer flush lengths for egg laying. The number of eggs laid on flush declines rapidly as the length of flush increases (Lin *et al*. 1973).

- Expanded leaves are not suitable sites for egg laying and thus gravid females (females filled with eggs) may migrate in response to a scarcity of suitable egg laying sites (Brlansky and Rogers 2007).
- *Diaphorina citri* eggs are small (0.31 mm in length) and would be difficult to detect during routine visual inspection (Aubert 1987b; EPPO 2005b).
- *Diaphorina citri* nymphs are small, flat and tend to wrap themselves around the shoot where they feed, which may make visual detection difficult (Aubert 1987b; Halbert and Manjunath 2004). First–fifth instar stages measure 0.25–0.35 mm, 0.49–0.53 mm, 0.69–0.72 mm, 0.98–1.05 mm and 1.45–1.58 mm in length, respectively (EPPO 2005b; Mead 2008).
- Adults of *D. citri* are 2.5 mm in length (EPPO 2009), and may be detected with the naked eye.
- The presence of large numbers of adults on the foliage of host plants increases the likelihood of the psyllids being associated with leaves during harvesting.
- Live adult psyllids were intercepted in shipments of fresh curry leaves (*Murraya koenigii*) from Hawaii to California (Wilkinson 2007; Filippini 2008). More recently, live nymphs and adults of *D. citri* have been intercepted on curry leaves, in unaccompanied baggage, from India and Texas into California (CDFA 2009; 2009; The Packer 2009; Associated Press 2009).
- Additionally, adult *D. citri* has been intercepted on non-host herbs *Moringa oleifera* Lam. (Brassicales: Moringaceae) and sweet basil leaf, *Ocimum basilicum* L. (Lamiales: Lamiaceae), from Hawaii to California (CDFA 2008b; Beattie and Barkley 2009) and on fresh coriander leaves, *Coriandrum sativum* L. (Apiales: Apiaceae), from Mexico to the USA (The Monitor 2009).

#### *Ability of the pest to survive transport and storage*

- *Diaphorina citri* is able to survive transport and storage as the insect is resilient to temperatures (Hall 2008b; Barkley and Beattie 2008). Fresh leaves are expected to be shipped at moderate temperatures and humidity levels that are unlikely to adversely affect any *D. citri* populations that are present. Furthermore, fresh leaves would provide an ample food supply during transit.
- *Diaphorina citri* has been intercepted on fresh curry leaves (Wilkinson 2007; Filippini 2008, CDFA 2009; 2009; The Packer 2009; Associated Press 2009), and non-host herbs (CDFA 2008b; Beattie and Barkley 2009; The Monitor 2009). This interception data indicates that this species is able to survive transportation and storage.

#### *Ability of the pest to survive existing pest management procedures*

- Chemicals are used to control the psyllid in citrus orchards in Réunion and mainland China (Aubert and Quilici 1984; Xu *et al*. 1991). Neem oil and petroleum spray oil have been trialled for use in India and China (Chakravarthi *et al*. 1998; Rae *et al*. 1997).
- The parasite, *Tamarixia radiata* has been used to control *D. citri* (Aubert *et al*. 1980). Other members of Syrphidae and Coccinellidae have been reported to feed on *D. citri*. These control methods will likely reduce, but not necessarily eliminate, *D. citri*.

The small size of the pest, the potential presence of all life stages in association with the commodity, tolerance of a range of temperatures and the interception of adult and juvenile stages of the psyllid on fresh leaves supports an assessment of 'high' for the importation of this species with fresh leaves.

## Probability of distribution

The likelihood that *Diaphorina citri* will be distributed within Australia, in a viable state with imported fresh leaves from countries where the insect is present, and transfer to a suitable host is **HIGH**.

#### *Ability of the pest to move from the pathway to a suitable host*

- Adult *D. citri* associated with imported fresh leaves are able to move independently from the pathway to a suitable host. Although they are considered weak fliers, adults have been recorded to regularly fly distances of 8–60 m (Hall 2008a) and have been estimated to be transported for 0.5–1 km on wind currents (Barkley and Beattie 2008).
- Eggs or nymphs arriving in Australia on fresh leaves would still need to develop into mature larvae and adults. The life cycle of this species varies from 15–47 days from egg to adult on live plants, and the adults survive for several months thereafter, depending on climatic conditions (Mead 2008).
- Nymphs are able to crawl short distances to suitable hosts if brought into close contact with another host plant (Barkley and Beattie 2008).
- The host range of Asian citrus psyllid includes 25 genera in the family Rutaceae, although not all of these are good hosts (Halbert and Manjunath 2004). Their preferred hosts are in the genera *Citropsis*, *Citrus* and *Murraya* (Grafton-Cardwell *et al.* 2006).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

#### *Distribution of the imported commodity in the PRA area*

- Fresh leaves will be distributed for all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption (e.g. curry leaves).
- Fresh leaves may be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the produce is likely to reach areas of host abundance.
- *Diaphorina citri* would need to survive transportation and storage within the PRA area. Fresh leaves are generally stored and transported at moderate temperatures and humidity to ensure the product remains fresh. Furthermore, fresh leaves would provide an ample food supply during distribution.
- Thus, *D. citri* present on fresh leaves distributed for retail or market sale, have the ability to survive transport and storage conditions and be distributed throughout the PRA area.

#### *Risks from by-products and waste*

- Although the intended use of fresh leaves includes all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption, waste material would be generated. Fresh leaves may be disposed of at multiple locations throughout Australia in compost bins or amongst general waste.
- Adults may be able survive on waste material for a short time before they disperse to suitable hosts. However, *D. citri* feed preferably on tender leaves and the tender portions of plant branches and succulent stems (Brlansky and Rogers 2007) and are unlikely to feed or survive for long on discarded waste.
- *Diaphorina citri* adults are able to survive from 52.5–94.5 hours without feeding (McFarland and Hoy 2001) and are able to move independently (Hall 2008a).
- Adult *D. citri* are also known to survive, without food or water for increasing periods as temperature decreases and humidity increases (McFarland and Hoy 2001). For example, at 25 °C, 50% mortality was observed at 7%, 33%, 53%, 73% and 97%

relative humidity for 24.9 hours, 28.3 hours, 28.5 hours, 37 hours and 43.2 hours, respectively (McFarland and Hoy 2001).

The ability for psyllids to disperse both independently and through the movement of infested fresh leaves and the presence of numerous host species in the PRA area supports an assessment of 'high' for this species.

### Overall probability of entry (importation x distribution)

The overall probability of entry for *Diaphorina citri* is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2). The overall probability of entry for the two pathways being assessed in this PRA is set out in Table 5.10.

**Table 5.10: Overall probability of entry of *Diaphorina citri* for on different pathways**

| Pathway | Probability of importation | Probability of distribution | Overall probability of entry |
|---|---|---|---|
| Fruit | Moderate | High | Moderate |
| Nursery stock (live plants) | High | High | High |
| Budwood | Low | High | Low |
| Fresh leaves | High | High | High |

## 5.4.2 Probability of establishment

The likelihood that *Diaphorina citri* will establish within Australia based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is **HIGH**.

***Availability of suitable hosts, alternative hosts and vectors in the PRA area***

- The host range of *D. citri* is mostly within the Aurantioideae subfamily of the Rutaceae with a preference for *Murraya paniculata* and *Citrus* species (Tsai and Liu 2000). However, host preference is influenced by season, variety, flush morphology, abundance, and frequency and duration of flushing.
- *Citrus* species are the host species of greatest concern, as the citrus industry is widely distributed throughout the PRA area. *Citrus* species are also used as street trees in some regions of the PRA area and are commonly planted in residential areas as domestic fruit trees.
- Other host species of concern include *Murraya paniculata* (Tsai and Liu 2000), which are sold as ornamental garden plants within the PRA area. Additionally *Murraya koenigii* is a host plant in India and Sri Lanka. This species is present in Australia but is not as commonly cultivated as *M. paniculata* (Beattie *et al*. 2008).

***Suitability of the environment***

- Members of the Diaphorineae family have an ecological preference for dry climates (Hollis 1987) and *D. citri* prefers a warmer, drier environment (Floyd and Krass 2006). *Diaphorina citri* is distributed in a wide range of geographic regions (Halbert and Manjunath 2004), many of which are climatically similar to parts of Australia (Peel *et al.* 2007).
- *Diaphorina citri* is considered invasive and has become established after being introduced to new environments. *Diaphorina citri* is found in tropical and subtropical Asia, Afghanistan, Saudi Arabia, Yemen, Réunion, Mauritius, parts of South and Central America, Mexico, the Caribbean and the USA (Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, South Carolina and Texas) (Halbert

and Núñez 2004; Halbert and Manjunath 2004; CDFA 2008a; Mead 2008; NAPPO 2009c).

- Adults of *D. citri* can survive a wide range of temperatures -7–45 °C (Barkley and Beattie 2008). However, it is better adapted to regions with high saturation deficits (high temperatures and low relative humidity) and not to regions with low saturation deficits (medium to high temperatures and high relative humidity) (Halbert and Manjunath 2004).
- In the laboratory, the nymphal stages of a population of *D. citri* from Japan were vulnerable to temperatures of less than 15 °C, with 100% mortality recorded for all instars reared below this temperature (Nakata 2006). Chinese and North American populations of *D. citri* had nymphal development limits closer to 10 °C (Nakata 2006).
- Yang *et al*. (2006) considered a mean daily temperature above 6.4 °C, in the coldest month of the year, as suitable for psyllid survival and activity the following spring. Based on information about the distribution of *D. citri* in China (Yang *et al*. 2006), it is unlikely that the lowest temperatures recorded in the citrus growing regions of Australia will prevent establishment of this psyllid in these regions.
- Temperature, relative humidity and rainfall influence the development of *D. citri* populations. Areas with higher rainfall, relative humidity and temperature are not suitable for *D. citri* (Aubert 1987b).
  - *Diaphorina citri* is commonly found from sea level to 1500 m above sea level in the south-western Arabian Peninsular. Temperatures at such elevations range from a maximum of 32–34 °C in summer to 2.5 °C in winter. The psyllid was absent in orchards higher than 1700–1800 m above sea level where occasional frosts occur (Aubert 1987b).
  - *Diaphorina citri* is not found in the province of Guangxi, China above 1300 m (Chau *et al*. 1979). The highest elevations recorded for *D. citri* is 1000 m in Java, Indonesia (Aubert *et al*. 1985), 650 m in Réunion (Aubert and Quilic 1984) and 1350 m in Nepal (Lama *et al*. 1987). In these countries, temperatures at these elevations range from 4–28 °C but are accompanied by high relative humidity and rainfall (Aubert 1987b).
  - Relative humidity close to saturation is not suitable for immature stages of *D. citri* because of severe fungal epizootics (Aubert 1987b). Where minimum daily relative humidity exceeds 87–90% nymphal mortality of 60–70% can be expected (Aubert 1987b).
  - Experimental work has shown that adult survival increases at both 25 °C and 30°C with increasing humidity to 97% (McFarland and Hoy 2001).
  - Monthly rainfall in excess of 150 mm is generally associated with low populations of *D. citri* due to eggs and nymphs being washed off leaf surfaces (Aubert 1987b).
- *Diaphorina citri* has been shown to successfully overwinter in the field where night temperatures dropped to -5 °C on several nights (Halbert and Manjunath 2004). Temperatures above 30 °C may shorten the longevity of psyllid adults (< 30 days) and lower their reproductive fitness (Liu and Tsai 2000). Impacts of climate on adult longevity vary, and adults can live for up to 6–9 months in regions with cool to cold winters (Husain and Nath 1927; Xie *et al*. 1988).
- The requirement of this species for new growth means that its breeding periods are usually restricted to the warmer months of the year (Gottwald *et al.* 2007). However, *Murraya paniculata* is a widespread host in warmer regions of the PRA area (Barkley and Beattie 2008), has more growth flushes than *Citrus* species, and can occur about six weeks earlier than citrus (da Graça 1991). The prolonged growth of new shoots

across host species and throughout most of the year gives *D. citri* greater opportunity for establishment and population growth.

- Populations of *D. citri* under natural conditions in its native southern Asia are most probably restricted in size and distribution by low saturation deficits, low rainfall, natural enemies, and scarce seasonal host-plant flush growth suitable for oviposition and development of nymphs (Beattie and Barkley 2009).
- The psyllids high fecundity, ability to flourish on non-water stressed plants in hot climates (in which summer temperatures frequently exceed 40 ºC) with high saturation deficits, and the ability of females to detect very young flush growth, reflect its adaptation to such environments (Beattie and Barkley 2009).
- Congeneric species *Diaphorina tryoni* is present in Australia, but not on the Rutaceae (DEWHA 2009). This demonstrates the suitability of the environment for at least one species of this genus.
- *Diaphorina citri* was detected in the Northern Territory in 1915, but was eliminated during the 1918–1922 citrus canker eradication program. There have been no detections of this pest since (Bellis *et al.* 2005).
- Climate modelling has predicted that all major citrus growing regions of Australia would have suitable climates favourable for *D. citri* (Barkley and Beattie 2008).

***Cultural practices and control measures***

- Oil sprays and most conventional insecticides have proven effective as control agents for *D. citri* in south-eastern and southern Asia (Gottwald *et al.* 2007).
- *Diaphorina citri* is an external feeder and existing pest management practices may impact on the establishment of this insect in Australia. This would be more likely in commercial orchards where external pests are routinely controlled. However, control measures are less likely to be applied in urban/residential environments and will be non-existent on common host species in natural environments.
- Biological control has been utilised to some effect. The use of the parasitic wasp *Tamarixia radiata* has proven partially effective after it was intentionally released in Florida. Other natural enemies of *D. citri* include some syrphids and chrysopids (Barkley and Beattie 2008; Mead 2008). However, these species are not present in Australia.
- Another major component of an effective control program is the removal of preferred alternative hosts of *D. citri*. This would involve the removal, for example, of any orange jasmine or *Murraya* species plants growing near citrus plantings, and especially any growing near citrus nurseries.

***The reproductive strategy and survival of the pest***

- *Diaphorina citri* reproduces via sexual reproduction only. After a single mating egg laying decreases after two weeks (Wenninger and Hall 2008). Continual oviposition of fertilised eggs is maximised by multiple matings throughout the life of the psyllid (Wenninger and Hall 2008).
- There is good behavioural evidence that female *D. citri* produce a volatile pheromone that attracts males (Wenninger *et al*. 2008). The ability of females to attract males with a volatile pheromone will increase the chances of a successful mating, even at low psyllid densities. Mated females do not lose their attractiveness to males (Wenninger *et al*. 2008) and will therefore ensure continual mating throughout the life of the psyllid, resulting in maximum reproductive output.
- The timing of reproduction is dependent on the emergence of new growth on the host plants (Halbert and Manjunath 2004). As a result, the time of highest abundance is

usually in the warmer months, when the flush of new growth most commonly appears on *Citrus* species (Gottwald *et al.* 2007). In Florida, densities of the psyllid peak in May, August and October through to December, which coincides with new flush growth in *Murraya paniculata* (Tsai *et al*. 2002).

- *Diaphorina citri* is active for the entire year. Initiation of oogenesis, and subsequent maturation of eggs within ovaries, is closely related to the presence of buds (Dai *et al*. 1982; Huang *et al*. 1999) and breeding activity is largely suspended when citrus trees are dormant (Waterhouse 1998).
- Adults commence mating to correspond with the flush of new growth on the host species. Oviposition takes place on this new growth, and is achieved by the female attaching her eggs to the tips of growing shoots, and on or between unfurling leaves (Mead 2008).
- Oviposition preferences reflect the need for the five nymphal instars to complete their development on immature growth. Thus, gravid females may migrate in response to a scarcity of suitable egg laying sites (Brlansky and Rogers 2007). Most eggs are laid within 14 days of new growth commencing (Lin *et al*. 1973).
- Varying egg laying capacities are reported in the literature. Aubert (2008) reports that female psyllids may lay between 1000–2000 eggs in a matter of three weeks. As many as 1900 eggs, averaging 630–1230, has been reported by Huang (1990), and as few as 180–520 eggs by Pande (1971), with numerous figures quoted in between (e.g. 800 eggs (Mead 2008); 807 eggs (Husain and Nath 1927). It is therefore highly likely that *D. citri* will survive and establish a population in the PRA area.
- Tsai and Liu (2000) report varying egg laying capacities on different hosts: means of 858, 626, 572 and 612, and maximum of 1378, 830, 818 and 994 on grapefruit, orange jasmine, rough lemon and sour orange seedlings under laboratory conditions, respectively.
- After the eggs are laid, eclosion takes an average of three days, but can require more or less time depending on temperature (Liu and Tsai 2000). There are five nymphal instars (Mead 2008), which are completed in 11–15 days (Halbert and Manjunath 2004).
- Development from egg to adult requires 15 days at 28 °C (Hall 2008a), but may be as long as 47 days depending upon food supply and ambient temperature (Knapp *et al*. 2006). Humidity, temperature and host plant effects play an important role in the survival and developmental rate of the psyllid. Optimal adult survival is at humidity levels above 53%, but there is significant survival at humidity levels as low as 7% (McFarland and Hoy 2001).
- High fecundity, availability of suitable flush and climatic conditions results in multiple generations per year, which creates the potential for rapid psyllid population growth (Mead 2008). The number of generations per year depends on regional climates. Nine generations are not uncommon, but 11–12 generations may be possible in the presence of suitable flush growth (Husain and Nath 1927). Atwal *et al*. (1970) reported 16 generations per year in Punjab, and Yang *et al*. (2006) reported 6–11 across several provinces in China.
- *Diaphorina citri* does not diapause, but population density decreases when citrus is not flushing because immature stages require new growth for development (Grafton-Caldwell *et al.* 2006).
- Adults of *D. citri* have a lifespan of several months, dependant on predation levels and the availability of food (Grafton-Cardwell *et al*. 2006).

- *Diaphorina citri* is able to overwinter on vegetation and possibly root stock in order to wait for more favourable breeding conditions (Gottwald *et al.* 2007) and will continue to feed throughout the colder months (Gottwald *et al.* 2007).

The suitability of the environment, presence of multiple host species throughout the PRA area, high reproductive potential and proven ability to establish in new regions supports an assessment of 'high' for the establishment of this species.

### 5.4.3 Probability of spread

The likelihood that *Diaphorina citri* will spread based on a comparison of factors in the area of origin and in Australia that affects the expansion of the geographic distribution of the pest is **HIGH**.

***The suitability of the natural or managed environment for natural spread***

- *Diaphorina citri* may have originated in India (Hollis 1987; Halbert and Manjunath 2004) and spread to south-east Asia, the Arabian Peninsula, North America, the Indian Oceans islands, South America (Halbert and Manjunath 2004) and the Caribbean (Grafton-Cardwell *et al*. 2006; CDFA 2008a) demonstrating its capacity to spread to similar environments.
- There are similarities in the natural and managed environments of the above regions with many of those in Australia, which suggests that *D. citri* could spread in Australia (Peel *et al.* 2007; Barkley and Beattie 2008).
- *Diaphorina citri* has been in Brazil since the 1940s (Michaud 2004). It later began to appear in other countries, and spread northwards, into Venezuela, the Caribbean islands, Central America, Mexico and the US states of Florida and Texas (Halbert and Manjunath 2004).
- The regions to which *D. citri* has recently spread and established (South America, North America and the Caribbean), have similar environments to Australia. The rate of spread was rapid, with the entire State of Florida being colonised within a decade. Invasion fronts moved on average up to12 miles (19 km) a year (Halbert *et al*. 2002; Gottwald *et al.* 2007).
- In Texas, *D. citri* was first recorded on orange jasmine in 2001 (French *et al*. 2001) and has now spread to 56 counties (da Graça *et al* 2008). Similarly, *D. citri* was detected in 2002 in the Yucatan Peninsula (Mexico) but subsequent surveys indicated that the psyllid is present in all citrus production areas of Mexico (Robles *et al.* 2008), demonstrating that *D. citri* is able to spread in the natural or controlled environment.
- Host plants that support the spread of *D. citri* are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (Barkley and Beattie 2008).
- *Diaphorina citri* feed and reproduce on members of the Rutaceae family, including cultivated *Citrus* species and Australian naturalised/native species, *Murraya paniculata* and *Citrus australasica* (Halbert and Manjunath 2004).
- *Citrus* production is widespread in Australia (Figure 1.1) and *Citrus* species and *Murraya paniculata* are popular as street trees, garden plants and ornamentals (Barkley and Beattie 2008).

- The similarities in climate between the current distribution of *D. citri* and citrus growing regions within Australia would suggest that this species could spread naturally in these areas (Barkley and Beattie 2008).

***Presence of natural barriers***

- The presence of natural barriers such as arid areas, mountain ranges, climatic differentials and possible long distances between hosts may prevent long-range natural spread of *D. citri*.
- *Diaphorina citri* is able to disperse independently. Although they are considered weak fliers, adults have been recorded to regularly fly distances of 8–60 m when searching for a host, although flights are generally limited to a few metres where hosts are abundant (Hall 2008a).
- There is circumstantial evidence that *D. citri* flew across the Florida Everglades and established in the eastern borders of large commercial citrus groves just to the west of the Everglades (Manjunath *et al*. 2008).
- Bass Strait may act as a natural barrier to *D. citri* from mainland Australia to Tasmania as the psyllids would not be capable of flying the distance over the strait. However, *D. citri* have the potential to move large distances on seasonal trade winds or hurricanes.
- Passive transport of *D. citri* through winds will help overcome natural barriers in Australia.
  - Field studies in the Philippines and China suggest possible medium to long distance transport of *D. citri* by strong winds in open orchards without windbreak protection (Aubert 1987b).
  - *Diaphorina citri* adults could to be taken into stratiform wind drifts and transported over distances ranging from 0.5–1 km (Aubert and Xia 1990) or 0.5–4 km (Aubert 1990).
  - Sakamaki (2005) speculated that migration of the psyllid in the Okinawan Islands in southeast Japan is governed by seasonal winds. It is possible for the psyllid to migrate 470 km northwards to the large island of Kyushu over sea by riding lower jet airstreams associated with summer monsoons.
  - Gottwald *et al*. (2007) speculated that *D. citri* may have been carried some 90–145 km by wind over non-citrus growing regions in Florida, and this long-distance movement may have been related to air masses during hurricanes or tropical storms.
- *Diaphorina citri* has been in South America since 1941 and has spread to Central America and the Caribbean. From here, it is believed to have been carried northward by seasonal trade winds or hurricanes to Florida (Tsai 2006). This suggests that adult psyllids may be able to overcome some natural barriers.
- The arid regions surrounding many *Citrus* species production areas in Australia would not prove to be a natural barrier to the spread of this pest in the presence of a host, as *D. citri* is heat tolerant (Bové 2006).
- However, the Australian citrus industry is spread across Australia in isolated regions. This will aid in containment and eradication of the vector if establishment occurs in an isolated citrus growing region (Barkley and Beattie 2008).
- *Diaphorina citri* will take advantage of any hot/dry season to multiply rapidly (Aubert 1988). These observations suggest that high summer temperatures in Australian citrus growing regions, which occasionally exceed 40 °C, will not limit the spread of *D. citri*.

- Areas with cold winters (with average daily winter temperatures of less than 6.4 °C) would act as a natural barrier due to the vulnerability of the nymphal stages to lower temperatures. Some evidence of this has been seen in the spread of *D. citri* in Japan; the species has not continued its range expansion past the northern tip of Okinawa Island despite its detection beyond this point. This is thought to be due to the increasingly cooler average winter temperatures (Nakata 2006).
- Adults of this species are tolerant of both high and low temperatures (-7–45 °C), and may therefore be less inhibited by climatic barriers (Barkley and Beattie 2008).
- Should *D. citri* be introduced to major commercial production areas of Australia physical barriers are unlikely to be a limiting factor to the spread as the psyllid has the potential to gradually spread by human activity to all fruit production areas in Australia.
- Where suitable host plants are not present it is likely that *D. citri* would migrate relatively short distances in search of suitable reproductive habitats (Barkley and Beattie 2008). Furthermore, the psyllids jump when disturbed, and then fly towards neighbouring plants.
- Psyllid migrations appear to be in response to increased competition for new host flush (Van den Berg *et al.* 1991b). Thus, natural spread is probably greatest in late spring and other periods when new flush is maturing and psyllid populations are high.

***Potential for movement with commodities or conveyances***

- Movement of host commodities would help the dispersal of *D. citri* because psyllids can be present on fruit, foliage, nursery stock and/or budwood. The major means of dispersal to previously uninfected areas is transport of infested propagative material (Halbert and Manjunath 2004).
- The most common mode of dispersal of *D. citri* is on plant parts transported by human activities. In particular, long-range dispersal occurs through the transport of infested plant material with 170 interceptions of live psyllids in the USA through 1985–2003 from Asia (Sullivan and Zink 2007).
- *Diaphorina citri* has been moved with plants for sale. For example, Halbert *et al.* (2002) speculates that trade in *Murraya paniculata* has distributed the psyllid, indicating that the means and pathways of movement of *D. citri* are diverse and common, and appear to have resulted in widespread distribution in the Florida peninsula and possibly to other states (Manjunath *et al*. 2008).
- Fruit is also considered a potential pathway for the movement of adults of *D. citri* (Gottwald *et al.* 2007; Barkley and Beattie 2008). Live *D. citri* have been intercepted on unprocessed fruit consignments shipped to Florida from the Bahamas (Halbert and Manjunath 2004).
- In Florida *D. citri* has been detected in trailers of harvested citrus fruit destined for processing (Halbert *et al.* 2010).
- *Diaphorina citri* has also been transported in empty fruit trucks in Parana State, Brazil (Beattie and Barkley 2009). Therefore it is likely that *D. citri* could disperse and move as a hitchhiker on trucks, trains, planes within Australia.

***Potential natural enemies***

- Several generalist predators such as spiders, lacewings, hover flies (or syrphids) and pirate bug feeds on *D. citri*. Coccinellid predatory beetles, *Olla v-nigrum* and *Harmonia axyridis*, are the most important predators of *D. citri* (Grafton-Cardwell *et al*. 2006). The use of the parasitic wasp *Tamarixia radiata* has been proven partially effective to control *D. citri* after it was intentionally released in Florida (Mead 2008; Barkley and Beattie 2008).

- The two parasites (*Tamarixia radiata* and *Diaphorencyrtus aligarhensis*) together may be partially effective to control *D. citri* (Floyd and Krass 2006). Biological control, however, may not be sufficient to adequately reduce insect populations, especially during the early spring months or in nurseries where trees are constantly putting on new growth (Floyd and Krass 2006).
- The specialist agents for the biological control of *D. citri* are not known to occur in Australia.

The suitability of the environment, presence of multiple host species throughout the PRA area, potential for spread in domestic commodities and conveyances, and their ability to disperse independently supports an assessment of 'high' for the spread of this species.

### 5.4.4 Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probabilities of entry, of establishment and of spread using the matrix of 'rules' for combining descriptive likelihoods (Table 2.2).

- The overall likelihood that *D. citri* will enter Australia by the pathways discussed in this PRA, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia are set out in Table 5.11 below.

**Table 5.11: Overall probability of entry, establishment and spread of *Diaphorina citri* by different pathways**

<table>
<tr><th rowspan="2">Pathway</th><th colspan="3">Probability</th><th rowspan="2">Overall probability of entry, of establishment and of spread</th></tr>
<tr><th>Entry</th><th>Establishment</th><th>Spread</th></tr>
<tr><td>Fruit</td><td>Moderate</td><td rowspan="4">High</td><td rowspan="4">High</td><td>Moderate</td></tr>
<tr><td>Nursery Stock (live plants)</td><td>High</td><td>High</td></tr>
<tr><td>Budwood</td><td>Low</td><td>Low</td></tr>
<tr><td>Fresh leaves</td><td>High</td><td>High</td></tr>
</table>

### 5.4.5 Consequences

*Diaphorina citri* is a vector of species of '*Candidatus* Liberibacter species', which cause HLB disease in *Citrus* species. The arrival and establishment of *D. citri* would mean the presence of a vector capable of spreading HLB disease caused by species of '*Ca.* L. species'.

- The consequences of the entry, establishment and spread of *Diaphorina citri* in Australia have been estimated according to the methods described in Table 2.3. The justification for these ratings is provided below.

| Criterion | Estimate and rationale |
|---|---|
| **Direct** | |
| Plant life or health | **Impact score**: **D** – Significant at the district level.<br>• *Diaphorina citri* damages plants directly through its feeding activities on new growth, causing stunting and malformation of leaves and twigs (Halbert and Manjunath 2004).<br>• New shoot growth that is heavily infested by the psyllid does not expand and develop normally, and is more susceptible to breaking-off (Grafton-Cardwell *et al*. 2006). Psyllids extract large quantities of sap from the plant as they feed and produce copious amounts of honeydew. The honeydew coats the leaves of the tree, encouraging sooty mould to grow (Grafton-Cardwell *et al*. 2006).<br>• *Diaphorina citri* nymphs inject a feeding toxin that causes considerable leaf and shoot distortion on citrus, often leading to the abatement of terminal elongation, and the abscission of leaves and whole shoots (Michaud 2004).<br>• *Diaphorina citri* is of primary concern because its establishment would mean the presence of a vector capable of spreading '*Ca.* L. species', which cause Huanglongbing (Bové 2006). Infected leaves show a mottled or blotchy appearance at the initial stage of symptom development. The yellowing spreads to other parts of the tree and dieback and rapid decline follow. Infected trees senesce within 3–5 years (Tsai 2006).<br>• Little direct economic loss has been reported due to the effects of *D. citri* alone (Tsai 2006). In Florida, *D. citri* is not considered to be a major problem in the absence of '*Candidatus* Liberibacter asiaticus' (Irey *et al*. 2008).<br>• Some Australian Rutaceae has been tested for susceptibility to '*Ca*. L. species' (Halbert and Manjunath 2004). Several native and naturalised species demonstrate disease symptoms following natural infection and many more species are suspected as being hosts, but have not undergone susceptibility trials. |
| Other aspects of the environment | **Impact score**: **C** – Significant at the local level.<br>There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>• In general, newly established species may affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants, degrade critical habitat or stimulate the use of chemicals or biological controls. There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>    – *Diaphorina citri* introduced into a new environment may compete for resources with native species and infest native Rutaceae species (Halbert and Manjunath 2004). |

| Criterion | Estimate and rationale |
|---|---|
| **Indirect** | |
| Eradication, control etc. | **Impact score**: **F** – Significant at the national level.<br>• History has demonstrated that once a psyllid capable of vectoring '*Ca.* L. species' arrives in a country the disease inevitably follows (Bové 2006).<br>• Due to the potential of the psyllid to vector an important disease, programs to eradicate this pest on host plants are likely to be costly and would include quarantine, pesticide applications and crop monitoring.<br>• Control of psyllid vectors is the key to limiting the impact of pathogen.<br>• A control program would add considerably to the cost of production of the host fruit. Area wide management of *D. citri* in Florida involves aerially applied dormant sprays in conjunction with biological controls (Qureshi and Stansly 2010).<br>• Though biological control is possible, effective control agents are not present in Australia. Introduction and establishment of such agents would require significant time and cost.<br>• Removal of native and naturalised host plants in the vicinity of citrus orchards or nurseries or the removal of infested citrus plants from orchards would be required to eradicate this psyllid.<br>• Considering the extreme fertility of the psyllid vector, with each female laying as many as 1000 to 2000 eggs in a matter of three weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment (Aubert 2008). |
| Domestic trade | **Impact score**: **D** – Significant at the district level.<br>• The presence of *D. citri* in commercial production areas and nurseries may result in interstate trade restrictions. Infested nurseries/orchards would be required to maintain a good vector control program, including removal of alternative hosts of the vector present on properties. This would increase the cost of production. |
| International trade | **Impact score**: **D** – Significant at the district level<br>• The presence of *D. citri* in commercial citrus production areas and nursery stock host areas could lead to limitations accessing overseas markets where these pests are absent. Infested nurseries/orchards would be required to maintain a good vector control program, including removal of alternative hosts of the vector present on properties. If the psyllid vector occurs in the area, citrus nurseries may be required to locate operations to greenhouses to protect plants from vectors. This would increase the cost of production. |
| Environmental and non-commercial | **Impact score**: **C** – Significant at the local level.<br>• Chemical treatments, removal of native hosts and plants infested with *D. citri* would have significant effects on the environment.<br>• With the exception of using neem extracts (Ahmed *et al.* 2004), there is no information on the availability of environmentally friendly pesticides for citrus psyllid control (Tsai 2006).<br>• Broad-scale chemical treatments may have some impacts on native insects.<br>• Direct application of insecticides may have some impact on water, soil and non-target organisms. |

Based on the decision rules described in Table 2.4, where the consequences of a pest with respect to one or more criteria are '**F**', the overall consequences are considered to be **HIGH**.

### 5.4.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for *D. citri* is shown below.

**Table 5.12: Unrestricted risk estimates of *Diaphorina citri* for different pathways**

| Pathway | Overall probability of entry, establishment and spread | Consequences | Unrestricted risk |
|---|---|---|---|
| Fruit | Moderate | High | High |
| Nursery stock (live plants) | High | | High |
| Budwood | Low | | Moderate |
| Fresh leaves | High | | High |

The unrestricted risk for *D. citri* has been assessed as 'high' for nursery stock, fruit and fresh leaves, and 'moderate' for budwood, which are all above Australia's ALOP. Therefore, specific risk management measures are required for this pest.

## 5.5 *Trioza erytreae*

*Trioza erytreae* (Del Guárico) is native to sub-Saharan Africa (Hollis 1984). It is the only species of *Trioza* known to feed and develop on the Rutaceae (Hollis 1984; Aubert 1987b), and belongs to a complex of species that are difficult to define morphologically, but which have discrete host plant preferences (Hollis 1984).

*Trioza erytreae* damages plants directly through its feeding activities on *Citrus* species and other Rutaceae on which they feed. The psyllid can cause severe leaf distortion, curling, stunting, galling and chlorosis (EPPO 2005a). The leaves may also be dusted with faecal pellets. These pellets of excrement (honeydew) have the appearance of minute white eggs, and the ground or vegetation under a badly-infested tree may appear as if dusted with white powder (Van der Merwe 1941). The severity of these symptoms is related to the level of infestations.

*Trioza erytreae* is heat-sensitive (Green and Catling 1971) and thrives only in cool environments (Bové 2006). The climatic range of *T. erytreae* is typically limited to temperate regions and is restricted to cooler highlands in countries of hot-arid to tropical climates (da Graça 1991; Bové 2006).

*Trioza erytreae* is the primary vector for '*Candidatus* Liberibacter africanus', which causes Huanglongbing (Van den Berg 1990; Bové 2006). In Réunion, *Trioza erytreae* is also a natural vector of '*Ca.* L. asiaticus' (Aubert 2008).

### 5.5.1 Probability of entry

### Pathway 1—Fruit

#### Probability of importation

The likelihood that *Trioza erytreae* will arrive in Australia with trade in fruit from countries where the insect is present is **MODERATE**.

***Association of the pest with the pathway***

- *Trioza erytreae* feeds and breeds on a wide range of plants within the Rutaceae (Moran 1968; Hollis 1984; Aubert 1987b). All life stages of *T. erytreae* are found on host foliage throughout its range (Van den Berg 1990).
- There are no records of *T. erytreae* laying eggs on mature fruits, nymphs completing their development on fruit, or nymphs and adults feeding on mature fruit. However, adult females occasionally lay eggs on young lemon fruit (Aubert 1987b; Van den Berg 1990).

- Eggs hatch in 6–15 days and nymphal development takes 17–43 days; development times are strongly correlated with mean temperature (Catling 1973). Citrus fruit maturation ranges from 6–7 months in the low tropics to 14–16 months in Mediterranean-type climates (Davies and Albrigo 1994). Therefore, eggs laid on immature fruit will have hatched and nymphs completed their development to the adult stage prior to fruit harvest.
- Although fruit is not an attractive feeding site for psyllids; foliage is an attractive site for oviposition, nymphal development, adult feeding and overwintering (Moran and Blowers 1967; Van den Berg 1990).
- The overwintering period for adults is likely to coincide with the harvest times for citrus fruits. The presence of large numbers of adults on the foliage of citrus trees in winter increases the likelihood of the psyllid being associated with fruit at harvest as hitchhikers.
- Consignments of citrus fruit with leaf material as a contaminant also presents a risk of entry of *T. erytreae*. Leaf material contaminating fruit may carry eggs or immature stages of the psyllid as eggs are laid on leaves and immature stages feeding on leaves are sessile (Moran and Blowers 1967; Van den Berg 1990; EPPO 2005a).
- Eggs and immature stages of *Trioza erytreae* eggs (0.28 mm) and nymphs (0.345–1.52 mm) are small and may escape visual detection.
- Adult male *T. erytreae* are 2.17 mm and adults females are 2.24 mm (Van den Berg 1990), and may be detected with the naked eye.
- Adult psyllids are attracted to bright lights (Barkley and Beattie 2008) and may therefore infest packing houses. Consequently, there is a risk of psyllids contaminating the fruit in packing houses.
- Although there is no information on the interception of *T. erytreae* on citrus fruit, Asian citrus psyllid (*D. citri*) has been intercepted on citrus fruit from psyllid infested areas (Halbert and Nunez 2004).

***Ability of the pest to survive transport and storage***

- Fruit destined for Australia would be shipped in refrigerated containers maintained at 4–6 ºC. These storage conditions are unlikely to have a significant impact on *T. erytreae* in imported fruit.
- The threshold temperature for nymphal development is 10–12 °C (Van den Berg 1990). Transport temperatures are not lethal to *T. erytreae* but are expected to slow development.
- However, feeding of nymphs is restricted to the young host tissues and there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990).
- *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988). The death of the psyllids under field conditions, where temperatures reached 27 °C and relative humidity dropped to 37%, was attributed to desiccation rather than starvation (Van den Berg and Deacon 1988). Therefore psyllids associated with fruit could survive for longer periods during transportation and storage under low temperatures and high humidity.

***Ability of the pest to survive existing pest management procedures***

- *Trioza erytreae* populations are suppressed in Southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006; Aubert 2008), and a range of generalist insect predators (Van den Berg 1990). These methods will reduce but not necessarily eliminate *T. erytreae*.

- Management measures applied to exported citrus from South Africa to the USA and lack of interception records for *T. erytreae* in citrus fruit consignments indicate the existing pest management procedures are working.

None of the life stages of this psyllid are directly associated with mature fruit; however, they may be associated indirectly as adult hitchhikers, or as eggs or nymphs on trash contaminating consignments. There is a lack of interception data for *T. erytreae* on fruit however; *Diaphorina citri* has been intercepted on citrus fruit from psyllid infested areas. Therefore an assessment of 'moderate' is given for the importation of this species in fruit commodities.

### Probability of distribution

The likelihood that *Trioza erytreae* will be distributed within Australia with fruit sourced from countries where the pest is present and be able to transfer to suitable hosts is **HIGH**.

#### *Ability of the pest to move from the pathway to a suitable host*

- Adult *T. erytreae* associated with imported fruit are able to move independently from the pathway to a suitable host. Although they are considered weak fliers, adult *T. erytreae* associated with imported fruit are able to move independently from the pathway to a suitable host. *Trioza erytreae* is able to disperse via wind currents up to 1.5 km (Van den Berg and Deacon 1988) and can survive up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Eggs or nymphs arriving in Australia on trash associated with imported fruit would still need to develop into mature nymphs and adult. Eggs hatch in about 5–17 days, nymphal development is completed in 17–43 days (Catling 1973) and adults have a lifespan of 2–3 months (Van den Berg 1990).
- However, feeding of nymphs is restricted to the young host tissues and there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990). There are no records of *T. erytreae* completing development from eggs to adult on trash or mature fruit.
- Nymphs of *T. erytreae* have limited dispersal capabilities. After hatching, the first instar nymph wanders about for a short time before settling down to insert its mouthparts and begin feeding (Moran and Blowers 1967).
- All life stages of *T. erytreae* are sensitive to high temperatures (above 32 °C) combined with low relative humidity (Moran and Blowers 1967). The eggs and first instars are considerably more vulnerable to these extremes than the more advanced stages (Catling 1969a). Climate may impact the ability of this pest to survive and move to a suitable host when distributed within the PRA area.
- *Trioza erytreae* feed and reproduce on the Rutaceae (Van den Berg 1990), including cultivated *Citrus* species and native/naturalised Australian species, *Murraya paniculata* and *Citrus australasica* (Van den Berg 1990; Aubert 1987b).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

#### *Distribution of the imported commodity in the PRA area*

- Fruit would be distributed to multiple destinations throughout Australia for retail sale. Therefore any adults or immature life stages associated with fruit would be distributed to multiple locations.
- *Trioza erytreae* would need to survive transportation and storage within the PRA area. The threshold temperature for nymphal development is 10–12 °C (Van den Berg 1990)

and minimum winter temperature in South Africa does not appear to cause significant mortality in *T. erytreae* (Catling 1967 as cited in Van den Berg 1990).
- After extended periods (> 60 days) without suitable foliage for oviposition *T. erytreae* can still oviposit hundreds of viable eggs once suitable foliage is located (Catling 1969a).
- Thus, *T. erytreae* present on fruit distributed for retail or market sale have the ability to survive transport and storage conditions and be distributed throughout the PRA area.

***Risks from by-products and waste***
- Although the intended use of fresh fruit is human consumption, waste material would be generated (e.g. overripe and damaged fruit, uneaten portions, trash). Whole or parts of the fruit may be disposed of at multiple locations throughout Australia in compost bins or amongst general household or retail waste.
- Adults of *T. erytreae* may survive on waste material for a short time, before dispersing to suitable hosts. However, *T. erytreae* feed preferably on new flush and are unlikely to feed or survive for long periods on discarded fruit. *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988), and are able to move independently (Aubert 1987b).
- Additionally humidity and temperature will play a role in the survival of *T. erytreae* in compost bins or amongst general waste. Optimal adult survival is at humidity above 25% and mean temperature between 18–30 °C (Buitendag and von Broembsen 1993).

The ability for psyllids to disperse both independently and through the movement of infested fruits, and numerous host species are present in the PRA area supports an assessment of 'high' for this species.

### Pathway 2—Nursery stock (live plants)

#### Probability of importation

The likelihood that *Trioza erytreae* will arrive in Australia with trade in nursery stock (live plants) from countries where the insect is present is **HIGH**.

***Association of the pest with the pathway***
- *Trioza erytreae* feeds and breeds on a wide range of plants within the Rutaceae (Moran 1968; Hollis 1984; Aubert 1987b), so nursery stock of host plants can be infested and provide a pathway for the importation of the psyllid into Australia.
- High psyllid population densities could be found in citrus nurseries, since the young trees are maintained in a state of almost constant growth (Floyd and Krass 2006). Active growth on alternate plant hosts support psyllid populations when citrus flush is not available (Floyd and Krass 2006).
- Adult females lay their eggs on the shoot tips of the youngest growth (Moran and Blowers 1967). Eggs are small (mean length 0.28 mm) and would be difficult to detect during routine visual inspection (Moran and Blowers 1967).
- *Trioza erytreae* nymphs are sedentary on leaves and stems where they are commonly found hiding away under young leaves (EPPO 2005a). First to fifth instar nymphs average 0.32 mm, 0.50 mm, 0.71 mm, 1.01 mm and 1.50 mm in length, respectively (Moran and Blowers 1967). These life stages would be difficult to detect during routine visual inspection.
- Adult male *T. erytreae* are 2.17 mm and adults females are 2.24 mm (Moran and Blowers 1967; Van den Berg 1990) and may be detected with the naked eye.

- *Trioza erytreae* adults have been reported to survive on semi-dormant plants (Van den Berg 1990). Therefore, live plants from infected areas may carry adults, eggs and/or nymphs over longer distances.

***Ability of the pest to survive transport and storage***

- Nursery stock is expected to be shipped at moderate temperatures and humidity levels, which are unlikely to adversely affect any *T. erytreae* populations that are present. The transport temperatures for citrus plant material are typically cool to cold and are unlikely to affect adult psyllid survival and may in fact prolong their life.
- *Trioza erytreae* been has been introduced into new areas through the importation of infested planting material (Bové 2006).
- Nursery stock can carry eggs and nymphs of *T. erytreae*. The nymphal stages associated with imported nursery stock are fairly sedentary and would need to complete their development into adults on their current host. In the case of nursery stock, this species has the potential to develop from eggs to nymphs during transport and storage as the host will provide ample food supply during this period.

***Ability of the pest to survive existing pest management procedures***

- *Trioza erytreae* numbers are often suppressed in southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006) and a range of generalist insect predators (Van den Berg 1990). These methods will reduce but not necessarily eliminate *T. erytreae*.

The small size of the pest, the association of all life stages with nursery stock and the likelihood of the species remaining active during transport supports an assessment of 'high' for the importation of this species in nursery stock.

### Probability of distribution

The likelihood that *Trioza erytreae* will be distributed within Australia in a viable state with imported nursery stock (live plants) from countries where the insect is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- *Trioza erytreae* arriving in Australia with nursery stock will not need to move from the import pathway to a suitable host as the psyllid is able to develop, reproduce and complete its life cycle without leaving the host.
- Natural movement of *T. erytreae* from infested nursery stock to a suitable host will be by passive transportation of adults in wind currents up to 1.5 km (Van den Berg and Deacon 1988).
- Eggs or nymphs arriving in Australia on nursery stock would still need to develop into mature larvae and adults. *Trioza erytreae* develops quickly (depending upon temperature), has a wide host range (Bové 2006) and adults are able to disperse independently and locate hosts with new shoot growth (Samways and Manicom 1983).
- Nymphs of *T. erytreae* also have limited dispersal capabilities. After hatching, the first instar nymph wanders about for a short time before settling down to insert its mouthparts and begin feeding (Moran and Blowers 1967). However, there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990).
- All stages of *T. erytreae* are sensitive to high temperatures (above 32 °C) combined with low relative humidity (Moran and Blowers 1967). The eggs and first instars are considerably more vulnerable to these extremes than the more advanced stages (Catling

1969a). Climate may impact the ability of this pest to survive and move to a suitable host when distributed within the PRA area.

- *Trioza erytreae* feeding and reproduction is largely confined to the Rutaceae family (Van den Berg 1990; Halbert and Manjunath 2004), including cultivated *Citrus* species and native/naturalised Australian species, *Murraya paniculata* and *Citrus australasica* (Van den Berg 1990; Aubert 1987b).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

***Distribution of the imported commodity in the PRA area***

- The distribution of nursery stock would be for retail distribution to multiple destinations throughout Australia. Nursery stock would also be distributed for commercial growing purposes.
- *Trioza erytreae* would need to survive transportation and storage within the PRA area. Nursery stock is expected to be maintained at moderate temperatures and humidity levels to ensure nursery stock survival, so a portion of infested propagation material that enters the country is likely to reach areas of host abundance.
- In the case of plant material for commercial use, if infested nursery stock was introduced, the psyllids could potentially move to other suitable host plants surrounding them, and be passively redistributed by the further movement of these infested plants.
- As nursery stock would be distributed throughout Australia and planted directly into suitable habitats there is no need for *T. erytreae* to be transported to a suitable host.

***Risks from by-products and waste***

- Although the intended use of live plants is propagation, waste material would be generated. Whole or parts of the plants may be disposed of at multiple locations throughout Australia in compost bins, amongst general household waste or through green waste centres.
- Any immature life stage of the psyllid associated with waste material would still need to complete their development into adults. However, *T. erytreae* require soft host material to feed (Van den Berg 1990).
- *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Additionally, humidity and temperature will play a role in the survival of *T. erytreae* in compost bins or amongst general waste. Optimal adult survival is at humidity above 25% and mean temperature of 18–30 °C (Buitendag and von Broembsen 1993).

The association of all life stages with the commodity, the likelihood of the species remaining active during transport and ability of adults to actively move from the commodity to a suitable host supports an assessment of 'high' for the distribution of this species in nursery stock.

## Pathway 3—Budwood

### Probability of importation

The likelihood that *Trioza erytreae* will arrive in Australia with trade in budwood from countries where the insect is present is **LOW**.

***Association of the pest with the pathway***

- *Trioza erytreae* feeds and breeds on a wide range of plants within the Rutaceae (Moran 1968; Hollis 1984; Aubert 1987b). Adults have been reported to survive on twigs and semi-dormant plants and nymphs are sedentary on leaves and stems (Aubert 1987b; Van den Berg 1990; EPPO 2005a).
- Budwood is typically sourced from older (e.g. one-year-old growth), foliage-free stems, with dormant buds suitable for grafting. Therefore, budwood is less likely to harbour eggs and feeding nymphs or adults as *T. erytreae* feeding and reproduction is closely linked with new leaf growth (Van den Berg 1990).
- *Trioza erytreae* eggs and nymphs are small and may escape visual detection. The average length of the egg is 0.28 mm, and the average lengths of the first–fifth instar nymphs are 0.32 mm, 0.50 mm, 0.71 mm, 1.01 mm and 1.50 mm, respectively (Moran and Blowers 1967).
- Adult male *T. erytreae* are 2.17 mm and adults females are 2.24 mm (Moran and Blowers 1967; Van den Berg 1990) and may be detected with the naked eye.

***Ability of the pest to survive transport and storage***

- Budwood is expected to be shipped at moderate temperatures and humidity levels that are unlikely to adversely affect any *T. erytreae* populations that are present. The transport temperatures for citrus plant material are typically cool to cold and are unlikely to affect psyllid survival and may in fact prolong their life.
- *Trioza erytreae* nymphs feeding on young growth in poor condition results in higher rates of mortality (Van den Berg 1990). As budwood is not the preferred feeding site for nymphs, the mortality rate of nymphs on budwood may also be high. However, adult *T. erytreae* can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).

***Ability of the pest to survive existing pest management procedures***

- *Trioza erytreae* numbers are often suppressed in southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006), and a range of generalist insect predators (Van den Berg 1990). These methods will reduce but not necessarily eliminate *T. erytreae*.

Although the preferred feeding sites for psyllids are new leaf and shoot growth, they may be associated with budwood at low levels and are likely to survive transport and storage. Therefore an assessment of 'low' for the importation of this species with budwood is given.

## Probability of distribution

The likelihood that *Trioza erytreae* will be distributed within Australia in a viable state with imported budwood from countries where the insect is present is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- Although they are considered weak fliers, adult *T. erytreae* associated with imported budwood are able to move independently from the pathway to a suitable host. *Trioza erytreae* is able to disperse via wind currents up to 1.5 km (Van den Berg and Deacon 1988) and can survive up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Eggs or nymphs arriving in Australia on imported budwood would still need to develop into mature nymphs and adult. Eggs hatch in about 5–17 days, nymphal development is

completed in 17–43 days (Catling 1973) and adults have a lifespan of 2–3 months (Van den Berg 1990).

- However, feeding of nymphs is restricted to the young host tissues and there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990). Nymphs of *T. erytreae* have limited dispersal capabilities. After hatching, the first instar nymph wanders about for a short time before settling down to insert its mouthparts and begin feeding (Moran and Blowers 1967).
- All stages of *T. erytreae* are sensitive to high temperatures (above 32 °C) combined with low relative humidity (Moran and Blowers 1967). The eggs and first instars are considerably more vulnerable to these extremes than the more advanced stages (Catling 1969a).
- *Trioza erytreae* feeding and reproduction is largely confined to the Rutaceae family (Van den Berg 1990; Halbert and Manjunath 2004), including cultivated *Citrus* species and native/naturalised Australian species, *Murraya paniculata* and *Citrus australasica* (Van den Berg 1990; Aubert 1987b).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

***Distribution of the imported commodity in the PRA area***

- Budwood is imported specifically for the purpose of grafting onto local rootstock and would be distributed to multiple destinations throughout Australia. Distribution of infested budwood commercially through nurseries would facilitate the distribution of *T. erytreae*.
- *Trioza erytreae* would need to survive transportation and storage within the PRA area. Budwood is expected to be maintained at moderate temperatures and humidity levels to ensure survival, so a portion of infested propagation material that enters the country is likely to reach areas of host abundance.
- After extended periods (> 60 days) without suitable foliage for oviposition *T. erytreae* can still oviposit hundreds of viable eggs once suitable foliage is located (Catling 1969a).

***Risks from by-products and waste***

- Although the intended use of budwood is for propagation, and all imported material is likely to be used, waste material may be generated. Whole or parts of the budwood may be disposed of at multiple locations throughout Australia as green waste or amongst general rubbish.
- Any immature life stage of the psyllid associated with waste material would still need to complete their development into adults. However, *T. erytreae* would require soft host material to feed (Van den Berg 1990).
- *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Additionally, humidity and temperature will play a role in the survival of *T. erytreae* in compost bins or amongst general waste. Optimal adult survival is at humidity above 25% and mean temperature of 18–30 °C (Buitendag and von Broembsen 1993).

The ability of *T. erytreae* to disperse both independently and through the movement of budwood, and numerous host species are present in the PRA area support an assessment of 'high' for distribution of this species with budwood.

## Pathway 4—Fresh leaves

### Probability of importation

The likelihood that *Trioza erytreae* will arrive in Australia with trade in fresh leaves from countries where the insect is present is **HIGH**.

#### *Association of the pest with the pathway*

- *Trioza erytreae* feeds and breeds on a wide range of plants within the Rutaceae (Hollis 1984) and its reproductive biology is closely tied to the availability of new leaf flush for egg laying and subsequent development of psyllid nymphs (Aubert 1987b; Van den Berg 1990; Bové 2006). Therefore, fresh leaves of host plants can carry eggs and nymphs and provide a pathway for the importation of the psyllid into Australia.
- Adults feed exclusively on the vegetation of the host plant (Van den Burg 1990) and lay their eggs on the shoot tips of the youngest growth (Moran and Blowers 1967). Nymphs are sedentary on leaves and stems where they are commonly found hiding away under young leaves (EPPO 2005a).
- *Trioza erytreae* eggs and nymphs are small and may escape visual detection. The average length of the egg is 0.28 mm, and the average lengths of the first–fifth instar nymphs are 0.32 mm, 0.50 mm, 0.71 mm, 1.01 mm and 1.50 mm, respectively (Moran and Blowers 1967).
- Adult male *T. erytreae* are 2.17 mm and adults females are 2.24 mm (Moran and Blowers 1967; Van den Berg 1990) and may be detected with the naked eye.
- The presence of large numbers of adults on the foliage of host plants increases the likelihood of the psyllids being associated with leaves during harvesting.
- Although there is no information on the interception of *T. erytreae* on fresh leaves of host plants but *Diaphorina citri* has been intercepted on fresh leaves of hosts (Wilkinson 2007; Filippini 2008) and non host species (CDFA 2008b; Beattie and Barkley 2009; The Monitor 2009).

#### *Ability of the pest to survive transport and storage*

- Fresh leaves are expected to be shipped at moderate temperatures and humidity levels which are unlikely to adversely affect any *T. erytreae* that are present. Furthermore, fresh leaves would provide an ample food supply during transit.
- Eggs will hatch in about 5–17 days and complete five nymphal instars in 17–43 days (Catling 1973). Both hatching and nymphal development are strongly correlated with mean temperature (Mead 1976) and there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990).

#### *Ability of the pest to survive existing pest management procedures*

- *Trioza erytreae* numbers are often suppressed in Southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006; Aubert 2008) and a range of generalist insect predators (Van den Berg 1990). These methods will reduce but not necessarily eliminate *T. erytreae*.

The pest is small in size, the potential presence of all life stages in association with the pathway and that it is likely to tolerate transport and storage conditions support an assessment of 'high' for the importation of this species with leaves.

### Probability of distribution

The likelihood that *Trioza erytreae* will be distributed within Australia, in a viable state with imported fresh leaves from countries where the insect is present and transferred to a suitable host is **HIGH**.

***Ability of the pest to move from the pathway to a suitable host***

- Adult *T. erytreae* associated with imported fresh leaves are able to move independently from the pathway to a suitable host. Although they are considered weak fliers, adults are able to disperse via wind currents up to 1.5 km (Van den Berg and Deacon 1988) and can survive up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Eggs or nymphs arriving in Australia on fresh leaves would still need to develop into mature nymphs and adult. Eggs hatch in about 5–17 days, nymphal development is completed in 17–43 days (Catling 1973) and adults have a lifespan of 2–3 months over winter (Van den Berg 1990).
- Nymphs of *T. erytreae* have limited dispersal capabilities. After hatching, the first instar nymph wanders about for a short time before settling down to insert its mouthparts and begin feeding (Moran and Blowers 1967). However, there is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990).
- All stages of *T. erytreae* are sensitive to high temperatures (above 32 °C) combined with low relative humidity (Moran and Blowers 1967). The eggs and first instars are considerably more vulnerable to these extremes than the more advanced stages (Catling 1969a). Climate may impact the ability of this pest to survive and move to a suitable host when distributed within the PRA area.
- *Trioza erytreae* feeding and reproduction is largely confined to the Rutaceae family (Van den Berg 1990; Halbert and Manjunath 2004), including cultivated *Citrus* species and native/naturalised Australian species, *Murraya paniculata* and *Citrus australasica* (Van den Berg 1990; Aubert 1987b).
- Suitable host species are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (ornamental and native Rutaceous species).

***Distribution of the imported commodity in the PRA area***

- Fresh leaves will be distributed for all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption.
- Fresh leaves may be distributed for retail or market sale to multiple destinations within the PRA area, so a portion of the produce is likely to reach areas of host abundance.
- *Trioza erytreae* would need to survive transportation and storage within the PRA area. Fresh leaves are generally stored and transported at moderate temperatures and humidity to ensure the product remains fresh. Furthermore, fresh leaves would provide an ample food supply during distribution.
- Thus, *T. erytreae* present on fresh leaves distributed for retail or market sale have the ability to survive transport and storage conditions and be distributed throughout the PRA area.

***Risks from by-products and waste***

- Although the intended use of fresh leaves includes all uses—other than as animal foods, fertilisers or for growing purposes—including human consumption, waste material would be generated. Fresh leaves may be disposed of at multiple locations throughout Australia in compost bins or amongst general waste.
- Adults may be able survive on waste material for a short time before they disperse to suitable hosts. However, *T. erytreae* feed preferably on new flush and are unlikely to feed or survive for long on discarded waste material.

- *Trioza erytreae* adults can live up to 55 hours without feeding if suitable foliage is not available (Catling 1973) or 85 hours in the absence of a suitable host plant in the field (Van den Berg and Deacon 1988).
- Additionally humidity and temperature will play a role in the survival of *T. erytreae* in compost bins or amongst general waste. Optimal adult survival is at humidity above 25% and mean temperatures of 18–30 °C (Buitendag and von Broembsen 1993). Field humidity and temperatures are unlikely to support long term survival of the psyllid in the absence of a host suitable for feeding.

The ability for psyllids to disperse both independently and through the movement of fresh leaves, and numerous host species are present in the PRA area supports an assessment of 'high' for this species.

### Overall probability of entry (importation x distribution)

The overall probability of entry for *Trioza erytreae* is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2). The overall probability of entry for the two pathways being assessed in this PRA is set out in Table 5.13.

**Table 5.13: Overall probability of entry of *Trioza erytreae* on different pathways**

| Pathway | Probability of importation | Probability of distribution | Overall probability of entry |
|---|---|---|---|
| Fruit | Moderate | High | Moderate |
| Nursery stock (live plants) | High | High | High |
| Budwood | Low | High | Low |
| Fresh leaves | High | High | High |

## 5.5.2 Probability of establishment

The likelihood that *Trioza erytreae* will establish within Australia based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is **HIGH**.

***Availability of suitable hosts, alternative hosts and vectors in the PRA area***

- *Trioza erytreae* also completes its development on *Citrus* species (Moran 1968; Aubert 1987b). *Citrus* species are the hosts of greatest concern in the PRA area, as the citrus industry is widely distributed throughout Australia.
- Some *Citrus* species are also used as street trees in regions of the PRA area and are commonly planted in residential areas as domestic fruit trees. Furthermore, studies indicate that young leaves of *Citrus limon* were significantly more attractive as feeding sites for adults than for South African indigenous host plants (Van den Berg 1990).
- Indigenous hosts of *T. erytreae* in Africa include *Clausena anisata*, *Fagara capensis* and *Vepris undulata* (Moran 1968; Hollis 1984; Aubert 1987b). *Trioza erytreae* also feeds on *Calodendrum capense*, but cannot complete its life cycle on this species (Moran 1968; Aubert 1987b). *Vepris mildbraediana* and *V. morogorensis* var. *subalata*, may be also be hosts of *T. erytreae* in Tanzania (Evers and Grisoni 1991; Temu and Andrew 2008). These hosts are not present in the PRA area.
- *Vepris undulata* is the preferred native host of *T. erytreae* (Moran 1968), which suggests that it may be the original host of this psyllid. *Clausena anisata* is the second-most favourable non-citrus host (Moran 1968). However, host preference is influenced by season, variety, flush morphology, abundance, frequency and duration of flushing (Catling 1971).

- Host quality may also impact the population of *T. erytreae* (Catling 1971). For example, nymphs that underwent prolonged development on poorly nourished citrus leaves, with poor conditions in the field had high rates of mortality, were flattened and reduced in size (Catling 1971).
- *Murraya paniculata* and *Clausena anisata* are of concern (Moran 1968) as these plants are sold as ornamental garden plants within the PRA area.

***Suitability of the environment***

- *Trioza erytreae* is adapted to relatively cool, higher altitude, low-saturation deficit environments (Catling 1969a; Green and Catling 1971). The species is reportedly sensitive to high temperatures combined with low humidity (Green and Catling 1971; Bové 2006).
- *Trioza erytreae* is distributed in a wide range of geographic regions (CABI/EPPO 2006; Van den Berg 1990), many of which are climatically similar to parts of Australia (Peel *et al.* 2007).
- The psyllid is considered invasive and has become established after being introduced to new environments. *Trioza erytreae* is established in a wide range of climates from the Cape Province, South Africa in the south to Eritrea in the north-east and Cameroon in the west (Van den Berg 1990). It is also reported from St. Helena, Mauritius, Madagascar, Réunion, Madeira (Portugal), Canary Islands (Spain), Saudi Arabia and Yemen (CABI/EPPO 2006). However, in these areas *T. erytreae* is only present in cool highland regions (Bové 2006).
- *Trioza erytreae* is limited across its range to temperate regions and is restricted to cooler highlands in countries of hot-arid to tropical climates (da Graça 1991; Bové 2006). Eggs and young instars are extremely vulnerable to desiccation and therefore this species would have trouble surviving arid to semi-arid climates with low rainfall and high temperatures (Aubert 1987b).
- *Trioza erytreae* cannot establish in hot and dry areas where midday temperatures regularly reach 32 °C or more combined with 30% RH (Catling 1969a; Aubert 1987b). If temperatures are above 32 °C for more than eight hours a day, eggs fail to hatch, there is increased mortality of all other life stages and ovarian development in the adult female is prevented (Moran and Blowers 1967).
- Beattie and Barkley (2009) reviewed the literature concerning the survival of *T. erytreae* and found that there is a consensus that *T. erytreae* is vulnerable to hot and dry conditions, particularly for eggs and early instar nymphs.
- The potential for populations to exist and flourish increases with increasing altitude. Population numbers are greatest in cool, moist regions up to 1280 m above sea level with low saturation deficits. Whereas, populations are small and isolated in the hot, arid lowlands (e.g. 500 m above sea level) with high saturation deficits (Beattie and Barkley 2009).
- Populations of this psyllid develop much better in cooler weather; hot summer days cause high mortality (Mead 1976).
- Five species of *Trioza* (*T. euginae, T. malloticola, T. pallida*, *T. oleariae* and *T. tristaniae*) occur in Australia, but not on the Rutaceae (DEWHA 2009). This demonstrates the suitability of the environment for at least some members of the genus.
- A model has been developed that uses temperature and humidity as parameters, which reliably predicts the known distribution of *T. erytreae*. Based on this information *T. erytreae* would have trouble establishing in the more arid to tropical regions of the PRA area. The southern regions of Australia are more likely to have climates suitable in most years for the establishment of *T. erytreae* (Barkley and Beattie 2008).

***Cultural practices and control measures***

- *Trioza erytreae* is an external feeder and existing pest management practices, such as routine pesticide applications, may impact the establishment of this insect in Australia. This would be more likely in commercial citrus orchards where external pests are routinely controlled. However, control measures are less likely to be applied in urban/residential environments and will be non existent on host species in natural environments.
- Removal of other hosts in the vicinity of citrus orchards may reduce the availability of breeding sites for the psyllids (Van den Berg 1990).
- *Trioza erytreae* populations are often suppressed in southern Africa by insecticidal sprays, the parasitoid wasps *Tetrastichus dryi* and *Psyllaephagus pulvinatus* (Pretorius and Van Vuuren 2006), and a range of generalist insect predators (Van den Berg 1990). The specialist parasitoids are not known to occur in Australia.

***The reproductive strategy and survival of the pest***

- *Trioza erytreae* is able to exploit its environment in a relatively short period of time because of extreme fecundity of the females, multiple generations per year, flying capacity and ability to build up on wild alternative Rutaceous host plants (Aubert 1987b).
- *Trioza erytreae* reproduces sexually and eggs of unmated females are infertile (Catling 1973). Eggs are laid on the shoot tips of the youngest growth and sometimes on tender young thorns, flower buds and young lemon fruit (Moran and Buchan 1975). Oviposition is completely suspended on dormant trees (Van den Berg 1990).
- The egg has a short stalk which is inserted into the leaf tissue. From the egg stage, *T. erytreae* undergoes five nymphal instars to reach the adult stage (Catling 1973).
- The life cycle of *T. erytreae* is temperature dependent and, on average, the total development time from egg to adult takes 22–62 days (Van den Berg 1990). Eggs may take 5–17 days to hatch, nymphal development (five instars) takes 17–43 days (Catling 1973) and adults survive for 2–3 in winter (Van den Berg 1990).
- The threshold temperature for nymphal development is probably between 10 °C and 12 °C (Catling 1973).
- Eggs and first instars are extremely vulnerable to temperatures above 25 °C (Evers and Grisoni 1991). Temperature also has an effect on population density, as high temperatures coupled with low humidity can cause high mortality in the nymph instars (Aubert 1987b).
- After hatching the first instar nymph wanders about for a short time before settling down to insert its mouthparts and begin feeding on young host tissues (Moran and Blowers 1967). There is often high mortality in the period between eclosion and settling of the crawlers (Van den Berg 1990).
- Following the final moult, mating may take place after three days in summer, and seven days in winter. Mating occurs at any time of day and females have been observed to mate 2–4 times a day (Van den Berg 1990). Mated females remain fertile for 11–16 days after mating (Catling 1973). Females will therefore require multiple matings to ensure viable eggs are laid throughout their life.
- Newly emerged females require a period of egg maturation before oviposition starts. This period is usually 3–5 days in summer (mean temperature 24–26 °C) and 6–7 days in winter (14–16 °C) (Catling 1973). However, in the absence of young flush the pre-oviposition period is extended and many females probably die without laying eggs (Van den Berg 1990).

- The role of pheromones in the reproductive biology of *T. erytreae* has been poorly studied (Van den Berg 1990). *Diaphorina citri* females are reported to produce a volatile pheromone that attracts males (Wenninger *et al*. 2008). Pheromones may increase the chances of attracting a mate, even at low psyllid densities.
- *Trioza erytreae* is active for the entire year. A female lays an average of 827 eggs during her lifetime (Van den Berg 1990). Mated females have been recorded to lay up to a maximum of 1305 eggs under laboratory conditions, whereas unmated females laid fewer eggs, which were infertile (Catling 1973).
- *Trioza erytreae* is capable of rapid population expansion when hosts with new shoot growth are available (Gottwald *et al* 2007). Under ideal conditions up to eight generations may occur in a year (Van den Berg 1990).
- The entire lifecycle of *T. erytreae* is completed on the host species (Van den Berg 1990). During winter, when conditions are not suitable for reproduction, the psyllid will continue to feed and survive until conditions become favourable for breeding (Aubert 1987b; Van den Berg 1990; Gottwald *et al* 2007).
- The population of *T. erytreae* is regulated by two density independent factors: flushing rhythm of hosts and the occurrence of, and sequences of, lethal weather extremes (Mead 1976).

The suitability of the environment, presence of multiple host species throughout the PRA area, high reproductive potential and proven ability to establish in new regions support an assessment of 'high' for establishment of this species.

### 5.5.3 Probability of spread

The likelihood that *Trioza erytreae* will spread based on a comparison of factors in the area of origin and in Australia that affects the expansion of the geographic distribution of the pest is **HIGH**.

***The suitability of the natural or managed environment for natural spread***

- *Trioza erytreae* is native to sub-Saharan Africa (Hollis 1984) and has spread to Madeira (Portugal), Canary Islands (Spain), Saudi Arabia and Yemen (CABI/EPPO 2006) indicating its ability to spread to similar environments.
- There are similarities in the natural and managed environments of the above regions with many of those in Australia, which suggests that *T. erytreae* could spread within Australia (Peel *et al.* 2007).
- The species has spread on the central, high plateau (1200–1500 m) in Madagascar, but not on low lying coastal areas. In Kenya, it is not reported below an altitude of 600–700 m (Bové 2006). Similarly, north of the Saudi Arabian/Yemeni border, *T. erytreae* occurs in cool highlands and is also only present in cool highlands of Réunion and Mauritius (Bové 2006).
- The preferred host (*Vepris lanceolata*) is not present in the PRA area. However, other hosts including *Citrus* species and *Murraya paniculata* are widespread in cities, towns and horticultural production areas throughout Australia and in the natural environment (Barkley and Beattie 2008).
- The effect of the natural environment on the spread of *T. erytreae* is best illustrated on Réunion Island in the Indian Ocean. Here *T. erytreae* co-exists with the other psyllid vector of HLB disease, *D. citri* (da Graça 1991). *Trioza erytreae* is restricted to altitudes above 500 m where the climate is cooler, while *D. citri* occurs below this altitude where the climate is warmer (da Graça 1991).

- The natural environment will impact on the spread of *T. erytreae* as this psyllid is heat-sensitive (Van den Berg 1990) and the eggs and first instars are extremely vulnerable to temperatures above 25 °C (Evers and Grisoni 1991). For example, *T. erytreae* cannot spread in dry areas where midday temperatures regularly reach 32 °C or more, combined with relative humidity at or below 30% (Catling 1969a; Aubert 1987b).
- Psyllid populations can increase rapidly under field conditions, particularly in spring when the nitrogen content of usually abundant flush growth is high and when competition between females for flush growth is low (Catling 1971). *Trioza erytreae* populations multiply rapidly in spring and early summer and less rapidly in autumn and winter (Catling 1971).
- Psyllid migrations appear to be in response to increased competition for new host flush (Van den Berg *et al.* 1991b). Thus, natural spread is probably greatest in late spring and other periods when new flush is maturing and psyllid populations are high.
- A model has been developed that uses temperature and humidity as parameters, which reliably predicts the known distribution of *T. erytreae*. Based on this information *T. erytreae* would have trouble establishing and spreading in the more arid to tropical regions of the PRA area. The southern regions of Australia are more likely to have climates suitable in most years for the spread of *T. erytreae* (Barkley and Beattie 2008).

***Presence of natural barriers***

- The presence of natural barriers such arid areas, mountain ranges, climatic differentials and possible long distances may prevent long-range natural spread of *T. erytreae*.
- Although *T. erytreae* is a weak flier, it is able to disperse independently over several hundred metres to locate hosts with new shoot growth (Samways and Manicom 1983). Van den Berg and Deacon (1988) report that adults are capable of dispersing 1.5 km in wind currents. Most flight activity takes place shortly before sunset and ceases with the onset of darkness (Van den Berg 1990). It has been observed that many of the dispersing psyllids spend the night on plants other than their normal Rutaceous hosts.
- *Trioza erytreae* has spread to Yemen, presumably from Ethiopia, across the Southern Red Sea, through the narrow "Bab al Mandab" strait, and has moved northwards (Bové 2006).
- Bass Strait may act as a natural barrier to *T. erytreae* from mainland Australia to Tasmania as the psyllids would not be capable of dispersing the distance over the strait. However, *T. erytreae* have the potential to move large distances (more than tens of kilometres) on seasonal trade winds or hurricanes (Bové 2006; Tsai 2006).
- Passive transport of *T. erytreae* through winds will help overcome natural barriers in Australia.
  - *T. erytreae* adults can disperse, with the aid of prevailing winds, at least 1.5 km (Van den Berg and Deacon 1988).
  - *Trioza erytreae* is thought to have spread from Madeira into the Canary Islands with the dominant North-South trade winds (Bové 2006).
- The arid regions surrounding many citrus production areas in Australia would prove to be a natural barrier to the spread of this pest. *Trioza erytreae* thrives only in cool environments, and is sensitive to high temperatures combined with low humidity (Green and Catling 1971; Aubert 1987b; Bové 2006).
- Should *T. erytreae* be introduced to major commercial production areas of Australia, physical barriers are unlikely to be a limiting factor to spread of this psyllid to temperate regions. *Trioza erytreae* has the potential to gradually spread by human activity to all fruit production areas in temperate Australia.

- The Australian citrus industry is spread across Australia in isolated regions. This will aid in containment and eradication of *T. erytreae* if establishment occurs in an isolated citrus growing region.

***Potential for movement with commodities or conveyances***

- Movement of commodities would help the dispersal of *T. erytreae* because psyllids can be present on fruit, fresh leaves, nursery stock and/or budwood. The major means of dispersal to previously uninfected areas is transport of infested propagation material (Bové 2006).

***Potential natural enemies***

- *Tetrastichus dryi*, *Tetrastichus sicarius* (eulophid ectoparasites) and *Psyllaephagus pulvinatus* (encyrtid endoparasite) have been observed as parasitoids of third to fifth instars of *T. erytreae* (Aubert 1987b). These natural enemies are not known to occur in Australia.
- In South Africa a range of generalist insect predators can suppress *T. erytreae* (Van den Berg 1990). In Australia, there are a number of generalist predators and, in commercial orchards, a range of pest control strategies that should limit *T. erytreae* (Beattie and Barkley 2009). These methods will reduce but not necessarily stop the spread of *T. erytreae*.

The suitability of the environment, presence of multiple host species throughout the PRA area, potential for spread in domestic commodities and conveyances and their ability to disperse independently supports an assessment of 'high ' for the spread of this species.

### 5.5.4 Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probabilities of entry, of establishment and of spread using the matrix of 'rules' for combining descriptive likelihoods (Table 2.2).

- The overall likelihoods that *Trioza erytreae* will enter Australia by the pathways discussed in this PRA, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia are set out in Table 5.14 below.

**Table 5.14: Overall probability of entry, establishment and spread of *Trioza erytreae* by different pathways**

| Pathway | Probability | | | Overall probability of entry, of establishment and of spread |
|---|---|---|---|---|
| | Entry | Establishment | Spread | |
| Fruit | Moderate | High | High | Moderate |
| Nursery Stock (live plants) | High | | | High |
| Budwood | Low | | | Low |
| Fresh leaves | High | | | High |

### 5.5.5 Consequences

*Trioza erytreae* is a vector of '*Candidatus* Liberibacter species', which cause HLB disease in *Citrus* species. The arrival and establishment of *T. erytreae* would mean the presence of a vector capable of spreading HLB disease.

- The consequences of the entry, establishment and spread of *T. erytreae* in Australia have been estimated according to the methods described in Table 2.3. The justification for these ratings is provided below.

| Criterion | Estimate and rationale |
|---|---|
| **Direct** | |
| Plant life or health | **Impact score**: **D** – Significant at the district level.<br>• *Trioza erytreae* attacks plants in the Rutaceae family (Van den Berg 1990). Leaves infested by the insect are distorted and often characterised by local chlorosis where the nymphs feed. Once the nymphs have attained maturity, the leaves assume their normal colour but not their natural shape (Catling 1973).<br>• Little direct economic loss has been reported due to the effects of this psyllid alone and it is considered a minor pest by itself (Van den Berg 1990).<br>• *Trioza erytreae* is a vector of '*Candidatus* Liberibacter species', which are known to cause HLB disease of citrus. Severely diseased trees are badly stunted, often sparsely foliated and are at times inclined to heavy leaf drop. Eventually severe tree decline occurs (Van den Berg 1990).<br>• *Trioza erytreae* is of primary concern because its establishment would mean the presence of a vector capable of spreading '*Ca.* L. species'. Some Australian Rutaceae, notably *Citrus australasica*, have been tested for susceptibility to '*Ca.* L. species' (Halbert and Manjunath 2004). |
| Other aspects of the environment | **Impact score**: **C** – Significant at the local level.<br>There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>• In general, newly established species may affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants, degrade critical habitat or stimulate the use of chemicals or biological controls. There may be some impact on insect or animal species that feed on host plants due to the reduced availability or vigour of these host plants.<br>– *Trioza erytreae* introduced into a new environment may compete for resources with native species and infest native Rutaceae species (Aubert 1987b; Van den Berg 1991). |
| **Indirect** | |
| Eradication, control etc. | **Impact score**: **F** – Significant at the national level.<br>• History has demonstrated that once a psyllid capable of vectoring '*Ca.* L. species' arrives in a country the disease inevitably follows (Bové 2006).<br>• Due to the potential of the psyllid to vector an important disease, programs to eradicate this pest on host plants are likely to be costly and would include quarantine, pesticide applications and crop monitoring.<br>• Control of psyllid vectors is the key to limiting the impact of pathogen.<br>• A control program would add considerably to the cost of production of the host fruit. Foliar sprays (Dimethoate, Endosulfan and Isofenphos) have been used in South Africa to control this psyllid (Van den Berg 1990).<br>• Though biological control is possible, effective control agents are not present in Australia. Introduction and establishment of such agents would require significant time and resources.<br>• Removal of native host plants, including naturalised species, in the vicinity of citrus orchards and the removal of infested citrus plants from orchards would be required to eradicate this psyllid. However, there is no control for '*Ca.* L. species'.<br>• Considering the extreme fertility of the psyllid vector, with each female laying as many as 1000 to 2000 eggs in a matter of three weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment (Aubert 2008). |
| Domestic trade | **Impact score**: **D** – Significant at the district level.<br>• The presence of *T. erytreae* in commercial citrus production areas and nurseries may result in interstate trade restrictions. Infested nurseries/orchards would be required to maintain a good vector control program, including removal of alternative hosts of the vector present on properties. This would increase the cost of production. |

| Criterion | Estimate and rationale |
|---|---|
| International trade | **Impact score**: **D** – Significant at the district level<br>• The presence of *T. erytreae* in commercial citrus production areas and nursery stock host areas could lead to limitations in accessing overseas markets where these pests are absent. Infested nurseries/orchards would be required to maintain a good vector control program, including removal of alternative hosts of the vector present on properties. If the psyllid vector occurs in the area, citrus nurseries may be required to locate operations to greenhouses to protect plants from vectors. This would increase the cost of production. |
| Environmental and non-commercial | **Impact score**: **C** – Significant at the local level<br>• Broad-scale chemical treatments, removal of native host and plants infested with *T. erytreae* and '*Ca.* L. species' would have significant effects on the environment.<br>• Broad-scale chemical treatments may have some impacts on native insects.<br>• Direct application of insecticides may have some impact on water, soil and non-target organisms. |

Based on the decision rules described in Table 2.4, where the consequences of a pest with respect to one or more criteria are '**F**', the overall consequences are considered to be **HIGH**.

### 5.5.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for *T. erytreae* is shown below.

**Table 5.15: Unrestricted risk estimates of *Trioza erytreae* for different pathways**

| Pathway | Overall probability of entry, establishment and spread | Consequences | Unrestricted risk |
|---|---|---|---|
| Fruit | Moderate | High | High |
| Nursery stock (live plants) | High | | High |
| Budwood | Low | | Moderate |
| Fresh leaves | High | | High |

The unrestricted risk for *T. erytreae* has been assessed as 'high' for fruit, nursery stock and fresh leaves, and 'moderate' for budwood, which are all above Australia's ALOP. Therefore, specific risk management measures are required for this pest.

## 5.6 Risk assessment conclusion

The results of the pathway risk assessments for '*Candidatus* Liberibacter species' and their vectors are set out in Table 5.16.

The unrestricted risk for '*Ca.* L. species' for the fruit and fresh leaves pathways have been assessed as 'very low', which meets Australia's ALOP. Therefore, specific risk management measures for fruit and fresh leaves are not required.

The unrestricted risk for nursery stock (live plants) and nursery stock budwood infected with '*Ca.* L. species' have been assessed as 'high' or 'low' in the absence of their psyllid vectors. The seed for sowing infected with '*Ca.* L. species' have been assessed as 'moderate' or 'low' in the absence of their psyllid vectors. Psyllids infected with '*Ca.* L. species' have been assessed as 'high'. These ratings exceed Australia's ALOP and therefore specific risk management measures are required to ensure that the bacterium does not enter, establish and spread though these pathways.

The unrestricted risk for fruit, nursery stock and fresh leaves infested with *D. citri* and *T. erytrae* have been assessed as 'high', and for nursery stock and for budwood the pathways have been assessed as 'moderate'. These ratings exceed Australia's ALOP and therefore specific risk management measures are required to ensure that the psyllids do not enter, establish and spread though these pathways.

**Table 5.16: Summary of pathway risk assessments for '*Candidatus* Liberibacter species and their vectors'**

| Pests/pathways | Entry | | | Establishment | Spread | P[EES] | Consequences | | | | | | | URE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Importation | Distribution | Overall | | | | Direct | | Indirect | | | | Overall | |
| | | | | | | | PLH | OE | EC | DT | IT | RNC | | |
| **'*Candidatus* Liberibacter africanus'** | | | | | | | | | | | | | | |
| Fruit (including seed) | VL | EL | **EL** | H | H (VL) | EL (EL) | E | B | F | D | D | C | H | VL (VL) |
| Nursery stock (live plants) | H | H | **H** | | | H (VL) | | | | | | | | H (L) |
| Budwood | M | H | **M** | | | M (VL) | | | | | | | | H (L) |
| Fresh leaves | M | EL | **EL** | | | EL (EL) | | | | | | | | VL (VL) |
| Seed for sowing | L | M | **L** | | | L (VL) | | | | | | | | M (L) |
| Infected psyllids | M | H | **M** | | | M | | | | | | | | H |
| **'*Candidatus* Liberibacter americanus'** | | | | | | | | | | | | | | |
| Fruit (including seed) | VL | EL | **EL** | H | H (VL) | EL (EL) | E | B | F | D | D | C | H | VL (VL) |
| Nursery stock (live plants) | H | H | **H** | | | H (VL) | | | | | | | | H (L) |
| Budwood | M | H | **M** | | | M (VL) | | | | | | | | H (L) |
| Fresh leaves | M | EL | **EL** | | | EL (EL) | | | | | | | | VL (VL) |
| Seed for sowing | L | M | **L** | | | L (VL) | | | | | | | | M (L) |
| Infected psyllids | M | H | **M** | | | M | | | | | | | | H |
| **'*Candidatus* Liberibacter asiaticus'** | | | | | | | | | | | | | | |
| Fruit (including seed) | VL | EL | **EL** | H | H (VL) | EL (EL) | E | B | F | D | D | C | H | VL (VL) |
| Nursery stock (live plants) | H | H | **H** | | | H (VL) | | | | | | | | H (L) |
| Budwood | M | H | **M** | | | M (VL) | | | | | | | | H (L) |
| Fresh leaves | M | EL | **EL** | | | EL (EL) | | | | | | | | VL (VL) |
| Seed for sowing | L | M | **L** | | | L (VL) | | | | | | | | M (L) |
| Infected psyllids | M | H | **M** | | | M | | | | | | | | H |

| **Pests/pathways** | **Entry** | | | **Establishment** | **Spread** | **P[EES]** | **Consequences** | | | | | | | **URE** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Importation | Distribution | **Overall** | | | | Direct | | Indirect | | | | **Overall** | |
| | | | | | | | **PLH** | **OE** | **EC** | **DT** | **IT** | **RNC** | | |
| ***Diaphorina citri*** | | | | | | | | | | | | | | |
| Fruit | M | H | M | H | H | M | D | C | F | D | D | C | H | H |
| Nursery stock | H | H | H | | | H | | | | | | | | H |
| Budwood | L | H | L | | | L | | | | | | | | M |
| Fresh leaves | H | H | H | | | H | | | | | | | | H |
| ***Trioza erytreae*** | | | | | | | | | | | | | | |
| Fruit | M | H | M | H | H | M | D | C | F | D | D | C | H | H |
| Nursery stock | H | H | H | | | H | | | | | | | | H |
| Budwood | L | H | L | | | L | | | | | | | | M |
| Fresh leaves | H | H | H | | | H | | | | | | | | H |

**Likelihoods for entry, establishment and spread**
**N** = Negligible
**EL** = Extremely low
**VL** = Very low
**L** = Low
**M** = Moderate
**H** = High
**P[EES]** = Overall probability of entry, establishment and spread

**Consequences**
Consequences from pest entry, establishment and spread are on an ascending scale from A to G (see method section 4).
**PLH** = Plant life or health
**OE** = Other aspects of the environment
**EC** = Eradication, control etc.
**DT** = Domestic trade
**IT** = International trade
**ENC** = Environnemental and non-commercial

**URE** = Unrestricted risk estimate

# 6 Pest risk management

Pest risk management evaluates and selects risk management options to reduce the risk of entry, establishment or spread of '*Candidatus* Liberibacter africanus', '*Ca.* L. americanus' and '*Ca.* L. asiaticus' and their vectors *Diaphorina citri* and *Trioza erytreae* for the pathways where the unrestricted risk exceeds Australia's ALOP.

The pathways identified for the introduction of '*Ca*. L. species' and their vectors *Diaphorina citri* and *Trioza erytreae* that have an unrestricted risk estimate above Australia's ALOP are presented in Table 6.1. Risk management measures are required to reduce this risk to achieve Australia's ALOP.

To effectively prevent the introduction of pests a series of important safeguards, conditions or phytosanitary measures must be in place. Australia currently has a well-established policy to import *Citrus* species for propagation (budwood, seed), fruit for consumption and fresh leaves of allied genera within the family Rutaceae. Currently, live plants of *Citrus* species and other allied genera in the Rutaceae family are not allowed entry into Australia. This draft pest risk analysis report supports the existing policy. Consistent with the existing policy, budwood, seed and fruit of *Citrus* species and fresh leaves of allied genera within the family Rutaceae would be subject to the existing measures to meet Australia's ALOP. Plant Biosecurity has evaluated the existing policy and proposed additional measures where required.

**Table 6.1 Proposed phytosanitary measures for '*Candidatus* Liberibacter species' and their vectors from all countries where these pests are present**

| Pathways | Pest type | Proposed additional measures |
|---|---|---|
| **Budwood** | ***Candidatus* Liberibacter species'**<br>• *Ca.* L. africanus'<br>• '*Ca.* L. americanus'<br>• '*Ca.* L. asiaticus' | • specific growth requirements and PCR testing before release from post-entry quarantine |
| **Rutaceous nursery stock** (live plants other than citrus species) | | • No live plants, budwood only; and<br>• specific growth requirements and PCR testing before release from post-entry quarantine |
| **Seed** (Citrus and all other host species) | | • Heat treatment and surface sterilisation **or** surface sterilisation and growth in post-entry quarantine facilities |
| **Fruit** | **Citrus psyllids**<br>• *Diaphorina citr*<br>• *Trioza erytreae* | • Area freedom* **or** Systems approach for fruit with pre- and post-harvest measures **or** Fruit treatment known to be effective against all life stages of the psyllid (e.g. methyl bromide fumigation) |
| **Fresh leaves** | | Existing policy is supported:<br>• Area freedom* **or** Fumigation **or** Heat treatment |
| * Area freedom may include pest free areas, pest free places of production or pest free production sites in accordance with ISPM 4 and ISPM 10. | | |

Plant Biosecurity considers that the risk management measures proposed in this pest risk analysis will achieve Australia's ALOP. While the following measures are proposed by

Plant Biosecurity, any other measure that provides an equivalent level of protection would be considered.

## 6.1 Existing risk management measures for propagative material, fruit and fresh leaves

### 6.1.1 Exiting policy to import *Citrus* species budwood

Australia's existing policy for budwood of *Citrus* species is based on tiered safeguards. This process ensures that if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed. Australia's existing policy on citrus nursery stock includes:

- budwood only to be imported
- mandatory on-arrival inspection
- mandatory methyl bromide fumigation
- mandatory sodium hypochlorite treatment by dipping
- mandatory heat treatment of budwood and
- mandatory growth in closed government PEQ facilities with pathogen screening

The existing PEQ arrangements for citrus budwood use a range of techniques to ensure freedom from pathogens. The techniques include: elimination treatments (surface sterilisation of budwood and shoot-tip-grafting), visual screening (for disease symptoms in PEQ) and active testing (biological indexing using woody indicators, serological testing using Enzyme-linked immuno-sorbent assay (ELISA) and molecular testing using Sequential-PAGE).

Budwood sourced from an AQIS-approved source may be exempt from some of the testing requirements provided that it is accompanied by the relevant documentation, including a phytosanitary certificate endorsed with the details of the virus status and source of the budwood; and an exporter's declaration clearly outlining the procedures adopted to screen the material for diseases, including details and dates of all indexing carried out, indicators/methods used, where the material has been held since indexing, and any other treatments applied. However, Plant Biosecurity recommends that budwood sourced from AQIS-approved sources should not be exempt from testing requirements for '*Ca.* L. species' and should be subjected to standard active testing.

**Mandatory on-arrival AQIS inspection**

All imported budwood requires mandatory on-arrival visual inspection to verify freedom from live insects. Citrus psyllids are small and infestations in the early instar stages in particular would be difficult to detect (Van den Berg 1990; Mead 2008). Similarly, latent infection caused by '*Ca*. L. species' can not be visually detected during inspection as clear visual signs of infection (particularly budwood sourced from recently infected plants) may not be present (Xu *et al*. 1988b; Wang *et al*. 1996; Gottwald *et al*. 2007). The delay in symptom expression and suppression of symptoms by high temperatures may mask the presence of the pathogen. Therefore, latent infections of '*Ca*. L. species' and infestation of budwood with early instar stages of the psyllid would not be detected during on-arrival visual inspection.

Reliance on on-arrival visual inspection only to detect pests is inefficient in the case of budwood. For this reason, visual inspection is not considered an appropriate measure to mitigate the risk posed by '*Ca*. L. species' and their vectors on budwood.

**Mandatory on-arrival fumigation**

Mandatory on-arrival fumigation is applied to minimise the risk of accidental introduction of arthropod pests. This risk management option is currently employed in Australia to reduce the risk of entry, establishment and spread of arthropod pests associated with *Citrus* species budwood.

Fumigation (e.g. with methyl bromide) is a treatment known to be effective against all life stages of *D. citri* and *T. erytreae*. For example, the USA is using methyl bromide as a measure to control *D. citri* as it is considered effective against all life stages (APHIS 2008a). Therefore, the existing requirement of mandatory fumigation for all shipments in accordance with the relevant AQIS standards is supported.

It is recommended that where methyl bromide fumigation of budwood is adopted, it must be completed in accordance with the relevant AQIS standards at one of the following rates:

- 48 $g/m^3$ for 2 hours at 10–15 °C
- 40 $g/m^3$ for 2 hours at 16–20 °C
- 32 $g/m^3$ for 2 hours at 21 °C +

Treatments for budwood, other than methyl bromide fumigation, will be considered on a case by case basis by Plant Biosecurity if proposed by an exporting country. Prior to the acceptance of an alternative fumigant Plant Biosecurity would have to assess the efficacy of that fumigant to ensure it gives an equal level of protection to methyl bromide.

The mandatory on-arrival fumigation will be effective against *D*. *citri* and *T*. *erytreae*. However, mandatory on-arrival fumigation may not be effective against phloem infecting '*Ca*. L. species'. Therefore additional risk management measures are required for '*Ca*. L. species'.

**Mandatory sodium hypochlorite treatment by dipping**

Mandatory on-arrival sodium hypochlorite treatment of budwood is applied for surface sterilisation. This risk management option is currently employed in Australia to reduce the likelihood of contamination with *Xanthomonas axonopodis* pv. *citri*.

The mandatory on-arrival treatment with sodium hypochlorite may not be effective against phloem infecting '*Ca*. L. species'. Therefore additional mitigation measures are required for '*Ca*. L. species'.

**Mandatory heat treatment**

Mandatory on-arrival heat treatment is applied to minimise the risk of accidental introduction of pathogens. This risk management option is currently employed in Australia to reduce the risk of entry, establishment and spread of pathogens associated with *Citrus* species budwood.

However, heat treatment (moist hot air or hot water) is not effective in eliminating '*Ca*. L. species' in budwood (Nariani *et al*. 1975; Cheema *et al.* 1982). Infected budwood, immersed in hot water at 45 °C for five hours gave no control and, at 50 °C for 30 minutes, two-thirds of the budwood treated developed symptoms (Nariani *et al*. 1975). When budwood was treated using hot moist air, with various combinations of temperature (45–51 °C) and time (1–6 hours) the pathogen was not eliminated (Nariani *et al*. 1975). The best treatment was 47 °C for four hours, which prevented HLB expression in 62% of treated budwood. For this reason, heat treatment is not considered an appropriate measure to manage '*Ca*. L. species' in budwood.

**Mandatory growth in closed government PEQ facilities with pathogen screening**

Mandatory growth of rootstock, grafted with imported budwood, in closed government PEQ facilities is applied to screen for pathogen freedom. Growing grafted rootstock in closed government PEQ facilities for a period of observation, and until the required pathogen screening/testing is completed, can increase the likelihood that pathogens will be detected. Therefore, the existing requirement of mandatory on-arrival growth in PEQ and pathogen elimination treatment, visual screening and active testing (biological indexing using woody indicators, serological testing using ELISA and molecular testing using Sequential-PAGE) is supported.

### 6.1.2 Proposed new measures to import *Citrus* species budwood

**Specific growth requirements for PEQ and PCR testing before release**

As symptom expression of HLB is influenced by temperature (Bové 2006; Gasparoto *et al*. 2008; Lopes *et al*. 2009a; Lopes *at al*. 2009b), Plant Biosecurity recommends that all imported plant material and indicator plants inoculated with imported material must be grown at appropriate temperatures to favour disease expression for all three HLB causing pathogens.

Studies indicate that '*Ca*. L. africanus' and '*Ca*. L. americanus' are heat-sensitive (Bové 2006; Lopes *et al*. 2009a) and do not produce symptoms above 27 °C. Temperatures in the range of 22–24 °C are optimum for the development of '*Ca.* L. africanus' (Bové 2006) and '*Ca.* L. americanus' (Gasparoto *et al*. 2008; Lopes *at al*. 2009a). '*Candidatus* Liberibacter asiaticus' is heat tolerant and produces symptoms above 27 °C (Bové 2006; Lopes *at al*. 2009b). Temperatures in the range of 17–32 °C are optimum for the development of '*Ca.* L. asiaticus' (Gasparoto *et al*. 2008; Lopes *at al*. 2009a). Therefore, during the PEQ period, it is proposed that plants be grown at 22–24 °C.

In addition to the observation for symptoms of '*Ca.* L. species' infection (see Section 4.1.1) and specific indexing, Plant Biosecurity proposes that an additional PCR test for '*Ca.* L. species' be added to this protocol. Polymerase chain reaction methodology has been developed to detect asymptomatic hosts when the bacterial titre is above a certain level (Li *et al*. 2008; Manjunath *et al*. 2008). Plant Biosecurity proposes that all plants of a line should be tested using available PCR primers specific to '*Ca.* L. species' at the end of the quarantine period, prior to release.

Under field conditions, where disease expression is influenced by the seasons, HLB symptoms may take two to three years to be expressed after initial infection (Beattie and Barkley 2009). However, under suitable conditions, young and actively growing hosts will show disease expression in a shorter period of time. After an extended period of host growth, under suitable conditions for HLB expression, testing of host tissue with PCR primers specific for '*Ca.* L. species' will be a final check to confirm the absence of the pathogen from imported nursery stock. The PCR test is important because asymptomatic hosts can have bacterial titres without disease expression.

The mandatory growth of plants propagated from imported budwood in closed quarantine until the required disease screening/testing is completed at 22–24 °C, and an additional PCR testing for '*Ca.* L. species' at the end of the quarantine period, will be effective in detecting '*Ca*. L. species'.

Plant Biosecurity recommends that the existing policy for the importation of *Citrus* species budwood with specific growth requirements of plants propagated from imported budwood (22–24 °C) and PCR testing before release from PEQ facilities be applied to manage '*Ca*. L. species'. This policy is based on tiered safeguards, which ensures that if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed.

### 6.1.3 Existing policy for nursery stock (live plants) of Rutaceous hosts

In addition to *Citrus* species, several allied genera within the family Rutaceae are known hosts of '*Ca*. L. species' and their vectors *D. citri* and *T. erytreae* (Appendix B). Therefore, nursery stock (live plants) of these hosts represents a similar risk to that of *Citrus* species. Currently, live plants of Rutaceous hosts are not allowed entry into Australia. The existing policy is supported.

Latent infection caused by '*Ca*. L. species' can not be visually detected during inspection as clear visual signs of infection (particularly in recently infected nursery stock) may not be present (Xu *et al*. 1988b; Wang *et al*. 1996; Gottwald *et al*. 2007). The delay in symptom expression and suppression of symptoms by high temperatures may mask the presence of the pathogen. Therefore, infected nursery stock would not be detected during on-arrival visual inspection.

Citrus psyllids are small and infestations in the egg and early instar stages in particular would be difficult to detect (Van den Berg 1990; Mead 2008). Nymphs are flat and tend to wrap themselves around the shoot where they feed, which may make visual detection difficult (Halbert and Manjunath 2004). First–fifth instar stages measure 0.25–0.35 mm, 0.49–0.53 mm, 0.69–0.72 mm, 0.98–1.05 mm and 1.45–1.58 mm in length, respectively (EPPO 2005b; Mead 2008). All imported nursery stock requires mandatory on-arrival fumigation to minimise the risk of accidental introduction of arthropod pests. The mandatory on-arrival fumigation will be effective against *D. citri* and *T. erytreae*. For example, the USA is using methyl bromide as a measure to control *D. citri* because it is considered effective against all life stages (APHIS 2008a). However, mandatory on-arrival fumigation may not be effective against phloem infecting '*Ca*. L. species'.

The risk of '*Ca*. L. species' entering Australia undetected would be increased by importing whole plants as the pathogen could be present and isolated in any part of the plant (Tatineni *et al.* 2008; Folimonova *et al.* 2009), and testing would prove difficult.

### 6.1.4 Proposed new measures to import Rutaceous hosts nursery stock

Plant Biosecurity proposes that the importation of Rutaceous hosts should be limited to budwood as this provides a specific part of the plant for testing and increases the chance of detecting '*Ca*. L. species'. That is, whole live plants would not be permitted.

Plant Biosecurity recommends that the existing policy for the importation of *Citrus* species budwood (mandatory on-arrival inspection; mandatory methyl bromide fumigation; mandatory sodium hypochlorite treatment by dipping; mandatory heat treatment; and mandatory growth in closed government PEQ facilities with pathogen screening) with specific growth requirements (22–24 °C) and PCR testing before release from PEQ be applied to the budwood of all known Rutaceous hosts of '*Ca*. L. species'. This policy is based on tiered safeguards, which ensures that if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed.

**Other options for *Citrus* species and live plants of Rutaceous hosts**

*Sourcing nursery stock from pest free areas*

Area freedom is a measure that might be applied to manage the risk posed by '*Ca.* L. species' and their psyllid vectors in nursery stock imported into Australia. The requirements for establishing pest free areas or pest free places of production are set out in ISPM No. 4: *Establishment of pest free areas* (FAO 1996) and ISPM No. 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

Before area freedom could be adopted as a phytosanitary measure it would be necessary for the exporting party to scientifically demonstrate the establishment, maintenance and verification of area freedom. Australia's evaluation and acceptance of this claim will be based on ISPM 4 or 10 guidelines (as appropriate) and must be consistent with Australia's ALOP. Failure to adequately establish, maintain or verify area freedom is likely to result in the presence of '*Ca*. L. species' and their psyllid vectors (*D. citri* and *T. erytreae*) on nursery stock.

However, surveys required to establish a pest free area may be hampered by the time-lag between infection and HLB symptom expression (Gottwald *et al*. 2007). For example, '*Ca*. L. asiaticus' was reported in 2004 in Brazil and 2005 in Florida (Bové 2006). It is speculated that the bacterium was present in Brazil and Florida and unrecognized for a number of years (~5–10 years) before it was detected (Bové 2006; Gottwald *et al*. 2007). Plants infected with HLB bacteria may be asymptomatic in the initial stages of infection and could be easily overlooked (Bové 2006). Initial symptoms of HLB can easily be confused with host plant disorders such as mineral deficiency (e.g. Zinc), winter yellows and naturally re-greened fruit in late summer and autumn (Beattie and Barkley 2009). Furthermore, host symptoms of other diseases (e.g. Citrus tristeza virus and Phytophthora root rot) can be initially confused with HLB (Beattie and Barkley 2009). For this reason, any proposal for area freedom status will need to be assessed by Plant Biosecurity on a case by case basis. In determining area freedom for '*Ca*. L. species' the presence or absence of the psyllid vectors will be important.

### 6.1.5 Existing policy to import seed of *Citrus* species seed for sowing

Australia has a well established policy to import *Citrus* species seed for sowing. However, seed of several allied genera within the family Rutaceae are currently not allowed entry into Australia. Consistent with the existing policy, citrus seed would be subject to the existing measures to meet Australia's ALOP. Plant Biosecurity has evaluated the exiting policy and proposed additional measures where required.

Australia's current legislation prohibits the importation of citrus seed for sowing (C6066) except in the circumstances where AQIS issues import permits that may specify phytosanitary measures to effectively manage quarantine risks. Many seeds are capable of transmitting seed-borne pathogens and hence growth in quarantine is required to prevent the introduction of exotic pathogens in this way.

To effectively prevent the introduction of pathogens associated with citrus seed a series of important safeguards, conditions, or phytosanitary measures must be in place. Australia's existing policy allows citrus seed for sowing from South Africa (C17777), Spain (C17776) and the USA (C17778) only.

Australia's existing policy on citrus seed includes:

- mandatory on-arrival inspection
- surface sterilisation of seed
- heat treatment or, if sensitive to heat, mandatory growth in closed Government PEQ facilities with pathogen screening

**Mandatory on-arrival AQIS inspection**

All imported citrus seeds require mandatory on-arrival inspection to verify freedom from regulated articles to minimise the risk of accidental introduction of soil inhabiting pests, live insects or other prohibited seeds. Soil, insects and weed seeds may be contaminants found with consignments of seeds and they could carry serious exotic pathogens. Therefore, the existing requirement of freedom from regulated articles of quarantine concern is supported.

However, mandatory on-arrival AQIS inspection will not be effective against '*Ca*. L. species' as seeds may not display any symptoms of infection. Therefore, additional measures are required to mitigate the risk posed by '*Ca.* L. species' in citrus seed for sowing.

**Mandatory surface sterilisation**

Mandatory on-arrival or pre-shipment surface sterilisation is applied to minimise the risk of accidental introduction of pathogens. This risk management option is currently employed in Australia to reduce the risk of entry, establishment and spread of *Xanthomonas axonopodis* pv. *citri*.

Treatment of citrus seed with 8-hydroxyquinoline sulfate (T9321); or 1% sodium hypochlorite (T9371) is applied for surface sterilisation. Treatments may be performed either pre-shipment or on-arrival in Australia. These risk management options are currently employed in Australia for citrus seed from the USA to reduce the likelihood of contamination with *Xanthomonas axonopodis* pv. *citri*. However, the treatment with 8-hydroxyquinoline sulfate or sodium hypochlorite may not be effective against phloem infecting '*Ca*. L. species'. Therefore additional risk management measures are required to mitigate the risk post by '*Ca*. L. species'.

**Mandatory heat treatment**

Mandatory on-arrival heat treatment is applied to minimise the risk of accidental introduction of pathogens. This risk management option is currently employed in Australia to reduce the risk of entry, establishment and spread of *Xylella fastidiosa*.

Heat treatment is currently applied to citrus seed from USA only to mitigate the risk of *Xylella fastidiosa*. This treatment is supported and might also manage the risk posed by '*Ca*. L. species' in seed.

'*Candidatus* Liberibacter species' are heat-sensitive. For example, '*Ca*. L. *africanus*' and '*Ca*. L. *americanus*' are sensitive to temperatures above 32 °C (Aubert 1987a; Bové *et al*.

2008; Lopes *et al*. 2009a), and '*Ca*. L. asiaticus' is sensitive to temperatures above 38 °C (Lopes *et al*. 2009a). Plant Biosecurity considers that heat treatment is effective in inactivating '*Ca*. L. species' in seeds. Plant Biosecurity therefore, recommends hot water treatment of seed at 50 °C for 20 minutes from all sources.

If seed is considered to be sensitive to the hot water treatment then imported seed must be grown in closed government PEQ.

**Growth in closed government PEQ facilities with pathogen testing**

Mandatory growth in closed government PEQ facilities for 24 months is applied to seed that is heat sensitive to screen for pathogen freedom. Growing imported seed in PEQ facilities will minimize the risk of introducing '*Ca.* L. species' into Australia. Seed must be surface sterilised (T9321 or T9371) before growing in the PEQ facilities to reduce the risk of accidental introduction of any pathogens. Symptom expression of HLB is influenced by temperatures (Bové 2006; Lopes *at al*. 2009a). Therefore, emerging seedlings must be grown at appropriate temperatures to favour disease expression in PEQ (refer to budwood PEQ requirements).

In addition to the observation of new growth for symptoms of '*Ca.* L. species' infection (see Section 4.1.1), Plant Biosecurity proposes that all plants should be actively indexed and tested using available PCR primers specific to '*Ca.* L. species' at the end of the quarantine period or when symptoms develop. Citrus has a specific indexing protocol (herbaceous plant) in PEQ and Plant Biosecurity proposes that an additional PCR test for '*Ca.* L. species' be added to this protocol. The PCR test is important because asymptomatic hosts can have bacterial titres without disease expression.

### 6.1.6 Proposed import policy for seed for sowing of all other known hosts

Several allied genera, predominantly within the family Rutaceae, are known hosts of '*Ca*. L. species' and their vectors (Appendix B). Therefore, seed of these genera represent a similar risk to that of *Citrus* species. Infected seed may represent an important pathway for introducing the bacterium to new areas as seed is imported for the specific purpose of propagation. To effectively prevent the introduction of '*Ca*. L. species' associated with seeds of host species, a series of important safeguards, conditions or phytosanitary measures must be in place. Therefore, it is recommended that the existing policy to import citrus seed is extended to the seeds of all known hosts of '*Ca*. L. species'. This includes:

- mandatory on-arrival inspection
- surface sterilisation of seed
- heat treatment or, if sensitive to heat, mandatory growth in closed Government PEQ facilities with pathogen screening

**Other options for seeds of Rutaceous hosts**

*Sourcing seed from pest free areas*

Area freedom is a measure that might be applied to manage the risk posed by '*Ca.* L. species' in seed imported into Australia. The requirements for establishing pest free areas or pest free places of production are set out in ISPM No. 4: *Establishment of pest free areas* (FAO 1996) and ISPM No. 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

Before area freedom could be adopted as a phytosanitary measure it would be necessary for the exporting party to scientifically demonstrate the establishment, maintenance and verification of area freedom. Australia's evaluation and acceptance of this claim will be based on ISPM 4 or 10 guidelines (as appropriate) and must be consistent with Australia's ALOP.

However, surveys required to establish pest free areas may be hampered by the time-lag between infection and HLB symptom expression (Gottwald *et al.* 2007). For example, '*Ca.* L. asiaticus' was reported in 2004 in Brazil and 2005 in Florida (Bové 2006). It is speculated that the bacterium was present in Brazil and Florida and unrecognized for several years (~5–10 years) before it was detected (Bové 2006; Gottwald *et al.* 2007). Plants infected with HLB bacteria may be asymptomatic in the initial stages of infection and could have been easily overlooked (Bové 2006). Initial symptoms of HLB can easily be confused with host plant disorders such as mineral deficiency (e.g. Zinc), winter yellows and naturally re-greened fruit in late summer and autumn (Beattie and Barkley 2009). Furthermore, host symptoms of other diseases (e.g. Citrus tristeza virus and Phytophthora root rot) can be initially confused with HLB (Beattie and Barkley 2009). For this reason, any proposal for area freedom status will need to be assessed by Plant Biosecurity on a case by case basis.

### 6.1.7 Existing policy to import citrus fruit for consumption

Australia has a well established policy to import *Citrus* fruit from Egypt (C9488, C9502), Israel (C6027), Italy (C10464), Spain (C6061, C6062) and the USA (C6109, C6026). In addition to these country specific conditions, all imports of citrus fruit are subjected to the general fruit and vegetable import requirements (C6000). The general requirements (C6000) include an AQIS import permit, a quarantine entry, a phytosanitary certificate, freedom from regulated articles and on-arrival inspection and remedial action by AQIS.

#### Mandatory on arrival inspection

All imported citrus fruit require either mandatory on-arrival inspection or pre-clearance inspection to verify freedom from regulated articles to minimise the risk of accidental introduction of live insects, disease symptoms, trash, contaminant seeds, soil and other debris. Live insects, trash, seeds, soil and other debris may be contaminants found with consignments of fruit and they could carry serious exotic pathogens. Therefore, the existing requirement of freedom from regulated articles of quarantine concern is supported.

Citrus fruit may be contaminated with adult psyllids and trash contaminating fruit may be infested with all life stages of the psyllids. Early instar stages in particular are small (Van den Berg 1990; Mead 2008) and would be difficult to detect. There have been interceptions of live adult *D. citri* at USA ports on fruit (Halbert and Manjunath 2004; Sullivan and Zink 2007). For this reason visual inspection alone is not considered an appropriate measure to verify whether the fruit is infested with psyllids. Therefore, additional measures would be required to mitigate the risk posed by the psyllids.

### 6.1.8 Proposed policy to import citrus fruit for consumption from countries with '*Ca.* L. species' and/or their vectors

#### Sourcing fruit from pest free areas

Area freedom is a measure that might be applied to manage the risk posed by psyllids in fruit imported into Australia. The requirements for establishing pest free areas or pest free places of production are set out in ISPM No. 4: *Establishment of pest free areas* (FAO 1996) and ISPM No. 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

Before area freedom could be adopted as a phytosanitary measure it would be necessary for the exporting party to scientifically demonstrate the establishment, maintenance and verification of area freedom. Australia's evaluation and acceptance of this claim will be based on ISPM 4 or 10 guidelines (as appropriate) and must be consistent with Australia's ALOP. Failure to adequately establish, maintain or verify area freedom is likely to result in the presence of *D. citri* and/or *T. erytreae* on fruit.

Any proposal for area freedom status will need to be assessed by Plant Biosecurity on a case by case basis.

**Systems approach**

Citrus production areas unable to meet the pest free area requirements may export citrus to Australia under a systems approach.

Plant Biosecurity proposes a systems approach to address the risks posed by citrus psyllids on citrus fruit. This approach is based on a combination of crop monitoring and psyllid control with post-harvest measures. The approach could be used to progressively reduce the risk of infected psyllids being imported to Australia with consignments of fruit. The elements of systems approach are:

*Controlling psyllid populations in the orchard*

*Diaphorina citri* is extremely fecund, with females laying as many as 1000–2000 eggs in a matter of three weeks (Aubert 2008). High fecundity, availability of new flush growth and suitable climatic conditions results in multiple generations per year, which creates the potential for rapid psyllid population growth (Mead 2008). The number of generations per year depends on regional climates. Nine generations are not uncommon, but 11–12 generations may be possible in the presence of suitable new flush growth (Husain and Nath 1927), for example, 16 generations per year in Punjab (Atwal *et al*. 1970) and 6–11 across several provinces in China (Yang *et al*. 2006) have been reported. Therefore monitoring and chemical control to reduce psyllid populations would be required to reduce the pest pressure on fruit entering the packinghouse.

*Post harvest processing*

Fruit may be contaminated with trash carrying early instar stages of the psyllid. Citrus fruit must therefore be subject to post-harvest processing including washing and brushing of the fruit to remove all life stages of the psyllid.

While a number of insect pests may be associated with citrus in the field and may remain associated with the fruit during harvest, packinghouse processes are likely to have a significant impact on whether these pests remain associated with citrus fruit after packing. The first significant step in post harvest processing is the removal of trash, grading, and washing to remove dirt and other external contaminants and commonly also remove the fruit's natural waxy layer. This step includes a combination of water or detergent sprays, coupled with brushed rollers gently scrub and wash the fruit as it passes. At the end of this process, the fruit is rinsed and dried. Fruit can then be graded, either manually or by electronic systems, before being coated with a 'wax' to protect the fruit.

For example, the USA the Code of Federal Regulations, Title 5, Part 51 (7 CFR 51) outlines post harvest procedures and provides a defined grading standard that must be met for fruit to be marketed under defined tolerance limits for the numbers of misshapen, damaged, or otherwise down-gradable fruit.

Of most interest is the removal of insects, especially psyllids, from fruit as a result of the combination of washing and brushing. As the aim of the process is to remove any dirt, spray residues and even sooty mould, it is unlikely that any insects could remain associated with the fruit following this process, providing this process was carried out effectively.

*Monitoring psyllids in the packing house*

Packinghouses will be required to monitor and control psyllids, and the storage areas will need to be secure to ensure fruit is not re-infested with psyllids after packing.

Citrus psyllids are strongly attracted to light and if packinghouses are located within infested areas, psyllids may contaminate the packinghouses. If packinghouses are not kept free from psyllids, it is likely that processed fruit may be reinfested by the psyllids.

There is anecdotal evidence that consignments may be contaminated with *D. citri* if packinghouses are infested with this pest. For example, live *D. citri* have been intercepted on fresh leaves of non-host plants *Moringa oleifera* [Moringaceae], *Ocimum basilicum* [Lamiaceae] (CDFA 2008b; Beattie and Barkley 2009) and *Coriandrum sativum* [Apiaceae] (The Monitor 2009). Therefore, packinghouses must be monitored and kept free of the psyllids to avoid infestation of citrus consignments destined for Australia.

Plant Biosecurity will consider the effectiveness of any systems approach proposed by exporting countries for their commodities.

**Treatment of fruit**

Citrus production areas unable to meet the systems approach requirements may export citrus to Australia under the mandatory fruit treatment protocol.

*Fumigation of fruit*

*Diaphorina citri* has been intercepted on fruit at USA ports (Halbert and Manjunath 2004; Sullivan and Zink 2007), which indicates that this pest is able to move with the commodity. Therefore, if fruits are sourced from psyllid infested areas it is highly likely that psyllids may be present as contaminants in the fruit. Fumigation (e.g. with methyl bromide) is a treatment known to be effective against all life stages of *D. citri* and *T. erytreae* and might be applied as a measure to manage the risk posed by psyllids associated with the importation of fruit.

It is proposed that where methyl bromide fumigation of fruit is adopted, it must be completed in accordance with the relevant AQIS standards at one of the following rates:

- 48 $g/m^3$ for 2 hours at 10–15 °C
- 40 $g/m^3$ for 2 hours at 16–20 °C
- 32 $g/m^3$ for 2 hours at 21 °C +

Treatments for fruit, other than methyl bromide fumigation, will be considered on a case by case basis by Plant Biosecurity if proposed by an exporting country. Prior to the acceptance of an alternative fumigant Plant Biosecurity would have to assess the efficacy of that fumigant to ensure it gives an equal level of protection to methyl bromide.

Treatments for fruit will need to be applied offshore to ensure that any live adult psyllids in consignments of fruit do not enter Australia.

### 6.1.9 Existing policy to import fresh leaves

Fresh citrus leaves are not permitted entry into Australia. Australia's existing policy permits the entry of fresh curry leaves (*Murraya koenigii*) from European countries, New Zealand, South Pacific Commission countries and the USA (California); and fresh bael leaves (*Aegle marmelos*) from New Zealand and South Pacific Commission countries, subject to specific import conditions. Both of these permitted species are hosts of *D. citri*.

Australia's existing policy for fresh leaves of *Murraya koenigii* and *Aegle marmelos* includes:

- mandatory on-arrival inspection, or pre-clearance by AQIS from all approved sources (C6000); and
- sourcing fresh leaves from pest free areas (New Zealand and USA); or
- mandatory on-arrival heat treatment at 85 °C for 8 hours (C6043) for *Murraya koenigii* leaves only (European countries and South Pacific Commission countries).

**Mandatory on-arrival AQIS inspection**

All imported fresh leaves of *Murraya koenigii* and *Aegle marmelos* require mandatory on-arrival visual inspection to verify freedom from live insects, unless pre-cleared by AQIS. This existing measure might be applied to verify whether fresh leaves are infested with psyllids. There have been interceptions of live *D. citri* on fresh curry leaves (*Murraya koenigii*), and on leaves of non-host herbs such as the kelor tree (*Moringa oleifera* [Moringaceae]) and sweet basil (*Ocimum basilicum* [Lamiaceae]) from Hawaii to California (CDFA 2008b; Beattie and Barkley 2009), and on fresh coriander (*Coriandrum sativum* L. [Apiaceae]), from Mexico to the USA (The Monitor 2009). However, citrus psyllids are small and infestations in the early instar stages would be difficult to detect (Van den Berg 1990; Mead 2008).

For this reason, visual inspection is not considered an appropriate measure to mitigate the risk posed by citrus psyllids on fresh leaves. Therefore, Australia's existing policy requires additional measures to mitigate the risk posed by citrus psyllids in fresh leaves.

**Sourcing fresh leaves from pest free areas**

Sourcing fresh leaves from citrus canker free areas is applied to address the risk of accidental introduction of this pathogen into Australia. This mitigation measure is supported and might also be applied to manage the risk posed by citrus psyllids. The requirements for establishing pest free areas or pest free places of production are set out in ISPM No. 4: *Establishment of pest free areas* (FAO 1996) and ISPM No. 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

Before area freedom could be adopted as a phytosanitary measure it would be necessary for the exporting party to scientifically demonstrate the establishment, maintenance and verification of area freedom. Australia's evaluation and acceptance of this claim will be based on ISPM 4 or 10 guidelines (as appropriate) and must be consistent with Australia's ALOP.

Any proposal for area freedom status will need to be assessed by Plant Biosecurity on a case by case basis.

**Heat treatment**

All imported *Murraya koenigii* leaves, from countries where citrus canker occurs, currently require mandatory heat treatment to manage the risk of citrus canker—imported consignments are hot air treated at 85 °C for 8 hours (T9569). This treatment is supported and might also be applied to manage the risk posed by all life stages of *D. citri* and *T. erytreae*. The objective of this risk management measure is to ensure that leaves exported to Australia are not infested with psyllids.

As all life stages of the psyllids are vulnerable to high temperature (Aubert 1987b), Plant Biosecurity considers that heat treatment will be effective in eliminating psyllid infestations on leaves. Hot air at not less than 85 °C for at least eight hours, once the core temperature has been reached, is proposed for fresh leaves imported from areas where citrus psyllids are present. For off-shore heat treatment, fresh leaves must be placed in secure packaging post-treatment to prevent re-infestations of the psyllid. For on-arrival treatment, fresh leaves would have to be in secure packaging, to prevent adult psyllids from dispersing before treatment.

**Other options for fresh curry leaves/bael leaves and other Rutaceous hosts**

*Fumigation of fresh leaves*

Live psyllids have been intercepted in shipments of fresh leaves of Rutaceous hosts and non-Rutaceous species (Wilkinson 2007; CDFA 2008b; Filippini 2008). Methyl bromide fumigation is a treatment known to be effective against all life stages of *D. citri* and *T. erytreae* and might be applied as a measure to manage the risk posed by psyllids associated with the importation of fresh leaves from citrus psyllid infested countries. For off-shore fumigation, fresh leaves must be in placed in secure packaging post-treatment to prevent re-infestations of the psyllid. For on-arrival treatment, fresh leaves would have to be in secure packaging, to prevent adult psyllids from dispersing before treatment.

It is proposed that where methyl bromide fumigation of fresh leaves is adopted, it must be completed in accordance with the relevant AQIS standards at one of the following rates:

- 48 $g/m^3$ for 2 hours at 10–15 °C
- 40 $g/m^3$ for 2 hours at 16–20 °C
- 32 $g/m^3$ for 2 hours at 21 °C +

Treatments for fresh leaves, other than methyl bromide fumigation, will be considered on a case by cases basis by Plant Biosecurity if proposed by an exporting country. Prior to the acceptance of an alternative fumigant Plant Biosecurity would have to assess the efficacy of that fumigant to ensure it gives an equal level of protection to methyl bromide.

## 6.2 Operational systems for the maintenance and verification of phytosanitary status of fruit

An existing operational system is in place for the imports of citrus fruit into Australia. This system would be sufficient to deal with the implementation of the measures proposed in this risk analysis.

### 6.2.1 Remedial action(s) for non-compliance

Should non-compliance with the import conditions be detected, the trade may be suspended or the import conditions amended until remedial action is completed and Plant Biosecurity and/or AQIS is satisfied that trade can recommence under the conditions set out in this pest risk analysis.

The objectives of this proposed procedure are to ensure that:

- any quarantine risk is addressed by remedial action, as appropriate
- non-compliance with import requirements is addressed, as appropriate.

# Conclusion

The findings of this qualitative, pest-initiated pathway risk analysis draft report are based on a comprehensive analysis of relevant scientific and other appropriate literature on '*Candidatus* Liberibacter africanus', '*Ca*. L. americanus' and '*Ca*. L. asiaticus' and their vectors *Diaphorina citri* and *Trioza erytreae*.

The pest risk analysis identified nursery stock (live plants), budwood, seed for sowing and psyllid vectors infected with '*Ca*. L. species' as potential pathways for the introduction of '*Ca*. L. species' into Australia. Fruit, nursery stock, budwood and fresh leaves were identified as potential pathways for the introduction of *D. citri* and *T. erytreae*. These potential pathways have an unrestricted risk that exceeds Australia's ALOP, therefore risk management measure are required.

Live plants of citrus and other allied genera in the Rutaceae family are not allowed entry into Australia. This draft pest risk analysis report supports the existing policy.

Plant Biosecurity considers that the risk management measures proposed in this draft PRA report will achieve Australia's ALOP against '*Ca*. L. species' and their psyllid vectors. Specifically, the proposed risk management measures are:

**For budwood (potentially carrying '*Ca.* L. species' and citrus psyllids):**

- extending existing policy for citrus budwood applies to all hosts of '*Ca*. L. species' (i.e., on arrival inspection, mandatory fumigation and mandatory growth in closed government PEQ facilities until the required pathogen screening/testing is completed); and
- additionally, plants grafted with imported budwood must be grown at 22–24 °C to promote symptom expression for '*Ca*. L. species' and PCR testing for '*Ca*. L. species' prior to release.

**For seed for sowing (potentially carrying '*Ca.* L. species'):**

- hot water treatment of seed at 50 °C for 20 minutes from all sources; or
- if seed is sensitive to heat treatment, mandatory surface sterilization and growth at 22–24 °C in closed government PEQ facilities for 24 months with pathogen screening including PCR testing for '*Ca*. L. species' prior to release.

**For fruit (potentially carrying citrus psyllids):**

- area freedom from citrus psyllids; or
- a systems approach using pre- and post-harvest measures to ensure that fruit is not infested with psyllids; or
- an application of a treatment to fruit known to be effective against all life stages of the psyllid (including but not limited to methyl bromide fumigation).

**For fresh leaves (potentially carrying citrus psyllids):**

- area freedom; or
- hot air treatment at 85 °C for 8 hours; or
- mandatory methyl bromide fumigation

Plant Biosecurity will consider any other measures suggested by stakeholders that provide an equivalent level of phytosanitary protection.

# Appendices

# Appendix A: Pest categorisation: pathway association for '*Candidatus* Liberibacter species' and their vectors

| Pest | Potential to be on Pathway | Present within Australia | Potential for establishment and spread | Potential for economic consequences | Consider further in the PRA |
|---|---|---|---|---|---|
| **DOMAIN BACTERIA** | | | | | |
| **Class Alphaproteobacteria** | | | | | |
| **Order Rhizobiales** | | | | | |
| *Candidatus* Liberibacter Africanus Jagoueix *et al*. 1994<br>*Candidatus* Liberibacter americanus Teixeira *et al*. 2005<br>*Candidatus* Liberibacter asiaticus Jagoueix *et al*. 1994 | **Yes:** potential pathways include fruit (Bové 2006), live plants, budwood (Aubert 1987a; Aubert 2008), fresh leaves (Tatineni *et al*. 2008), seed (Li *et al*. 2009; Tatineni *et al*. 2008) and infected psyllids (Aubert 2008) | No | **Yes:** the availability of suitable hosts (APNI 2008) and climate (Peel *et al.* 2007) will facilitate the establishment of '*Ca.* L. species' in the PRA area. The movement of infective propagative material will facilitate the spread of these bacteria. | **Yes:** Huanglongbing caused by '*Ca.* L. species' is a destructive disease of citrus. It is the major limiting factor for citrus production worldwide (da Graça 1991; Bové 2006; Aubert 2008). | **Yes** |
| **ARTHROPODA: Insecta** | | | | | |
| **Order Hemiptera** | | | | | |
| *Diaphorina citri* Kuwayama [Psyllidae] (synonym: *Euphalerus citri)* | **Yes:** potential pathways include fruit (Halbert and Núñez 2004), live plants (Halbert and Manjunath 2004; Sullivan and Zink 2007), budwood (EPPO 2009) and fresh leaves (Wilkinson 2007; Filippini 2008). | No | **Yes:** availability of host species (Tsai and Liu 2000; Beattie *et al*. 2008) and suitable conducive climate (Hollis 1987; Floyd and Krass 2006) will facilitate the establishment of *D. citri*. Both natural and human-assisted factors will aid in the spread of the psyllid (Halbert and Manjunath 2004; Halbert *et al*. 2002; Gottwald *et al.* 2007). | **Yes:** *Trioza erytreae* attacks plants in the Rutaceae family (Van den Berg 1990). Leaves infested by the insect are distorted and often characterised by local chlorosis where the nymphs feed (Catling 1973). *Trioza erytreae* is of primary concern because its establishment would mean the presence of a vector capable of spreading '*Ca.* L. species'. | **Yes** |

| Pest | Potential to be on Pathway | Present within Australia | Potential for establishment and spread | Potential for economic consequences | Consider further in the PRA |
|---|---|---|---|---|---|
| *Trioza erytreae* (Del Guercio) [Triozidae] (synonyms: *Spanioza eritreae* Del Guercio, *Aleurodes erytreae* Del Guercio, *Spanioza erythreae* Del Guercio, *Spanioza erytreae* Del Guercio, *Trioza citri* Laing, *Trioza erythreae* Del Guercio, *Trioza merwei* Pettey) | **Yes:** potential pathways include fruit, live plants (Moran 1968; Hollis 1984; Aubert 1987b), budwood (EPPO 2005a) and fresh leaves (Van den Berg 1990). | No | **Yes:** availability of host species (Beattie *et al.* 2008) and suitable climate (Catling 1969a; Green and Catling 1971; Peel *et al.* 2007; Barkley and Beattie 2008) will facilitate the establishment of *T. erytreae*. Both natural and human-assisted factors will aid in the spread of the psyllid (Van den Berg and Deacon 1988; Bové 2006). | **Yes:** *Diaphorina citri* damages plants directly through its feeding activities on new growth, often leading to the abatement of terminal elongation, and the abscission of leaves and whole shoots (Michaud 2004). *Diaphorina citri* is of primary concern because its establishment would mean the presence of a vector capable of spreading '*Ca.* L. species'. | **Yes** |

# Appendix B: Known hosts of '*Candidatus* Liberibacter species' and their psyllid vectors†

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| **Rutaceae [Aurantioideae: Aurantieae]** | | | | | | |
| *Aegle marmelos* (L.) Corrêa | | | | | Feeding (Beattie and Barkley 2009) | |
| *Aeglopsis chevalieri* Swingle | | | | Symptomless. Questionable host (Beattie and Barkley 2009) | Survival for five weeks (Beattie and Barkley 2009) | |
| *Afraegle gabonensis* (Swingle) Engl. | *Afraegle gabonensis* Engl. | | | | Nymphs (Beattie and Barkley 2009) | |
| *Afraegle paniculata* (Schumach.) Engl. | | | | | Nymphs and eggs (Beattie and Barkley 2009) | |
| *Atalantia buxifolia* (Poir.) Oliv. | *Severinia buxifolia* (Poiret) Ten. | | | Minor symptoms; no effect on growth (Beattie and Barkley 2009) | Survives and propagates (Beattie and Barkley 2009) | |
| *Atalantia monophylla* (L.) Corr. | | | | | Adults (Beattie and Barkley 2009) | |
| *Balsamocitrus dawei* Stapf. | | | | Symptoms (Beattie and Barkley 2009) | Survival for seven weeks (Beattie and Barkley 2009) | |
| *Burkillanthus malaccensis* (Ridl.) Swingle | | | | Host (FDA 2008) | | |
| *Citroncirus webberi* J. Ingram & H. E. Moore | | | | Symptoms including stunting and seed abortion (Beattie and Barkley 2009) | | |
| *Citropsis articulata* (Willd. ex | | | | | Good to moderate | |

| Host Species | Host name as cited by the original author | 'Candidatus Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| Spreng.) Swingle & M. Kellerm. | | | | | host (Beattie and Barkley 2009) | |
| *Citropsis gilletiana* Swingle & M. Kellerm. | | | | | Eggs, nymphs and adults; high numbers (Beattie and Barkley 2009) | |
| *Citrus amblycarpa* (Hassk.) Ochse | *Citrus amblycarpa* Ochse | | | Symptoms including stunting (Beattie and Barkley 2009) | Eggs (Beattie and Barkley 2009) | |
| *Citrus australasica* F. Muell | *Microcitrus australasica* (F. J. Muell.) Swingle | | | Stunting (Beattie and Barkley 2009) | Adult feeding and survival for five weeks (Beattie and Barkley 2009) | Feeding (Beattie and Barkley 2009) |
| | *Microcitrus australasica* (sic) | | | | Full development in lab (Beattie and Barkley 2009) | |
| *Citrus cavaleriei* H. Léveillé ex Cavalerie | *Citrus ichangensis* Swingle | | | Symptoms (Beattie and Barkley 2009) | Slight damage (Beattie and Barkley 2009) | |
| *Citrus hystrix* DC | | | | Symptoms (Beattie and Barkley 2009) | Good host (Beattie and Barkley 2009) | |
| | *Citrus macroptera* Montrouz. | | | Host (Beattie and Barkley 2009, Das and Kumar 2010) | | |
| *Citrus australis* (Mdie) Planch. | *Microcitrus australis* (Planch.) Swingle | | | | Slight damage; adults? (Beattie and Barkley 2009) | |
| *Citrus glauca* (Lindl.) Burkhill | *Eremocitrus glauca* (Lindl.) Swingle | | | | Survival for four weeks (Beattie and Barkley 2009) | |
| *Citrus glauca* x Shakura *Citrus reticulata* | *Eremocitrus* hybrid | | | | Damage and eggs (Beattie and | |

| **Host Species** | **Host name as cited by the original author** | **'*Candidatus* Liberibacter species'** | | | **Psyllid vector** | |
|---|---|---|---|---|---|---|
| | | **Africanus** | **Americanus** | **Asiaticus** | ***Diaphorina citri*** | ***Trioza erytreae*** |
| | | | | | Barkley 2009) | |
| *Citrus inodora* F. M. Bailey | | | | | Slight damage (Beattie and Barkley 2009) | |
| *Citrus japonica* Thunb | *Fortunella* spp. | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | Feeding and oviposition (Beattie and Barkley 2009) |
| | *Fortunella crassifolia* Swingle | | | | Good host (Beattie and Barkley 2009) | |
| | *Fortunella hindsii* (Champ. ex Benth.) Swingle | | | Cryptic symptoms (Beattie and Barkley 2009) | Adults to host (Beattie and Barkley 2009) | |
| | *Fortunella japonica* (Thunb.) Swingle | | | Cryptic symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Fortunella margarita* (Lour.) Swingle | | | Symptoms (Tsai *et al.* 2006) | Nymphs (Beattie and Barkley 2009) | |
| | *Citrus japonica* Thunb | | | | Few nymphs and adults (Beattie and Barkley 2009) | |
| | *Fortunella polyandra* (Ridley) Tanaka | | | | Eggs, nymphs and adults (Beattie and Barkley 2009) | |
| *Citrus maxima* (Burman) Merr. | *Citrus grandis* (L.) Osbeck | | | Symptoms (Beattie and Barkley 2009) | Moderate host (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) |
| | *Citrus maxima* (Burman) Merr. | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Citrus* species (pomelo) | | | Symptoms (Beattie and Barkley 2009) | | |
| | Pomelo | | | Symptoms (Beattie and Barkley 2009) | | |

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| | *Citrus obovoidea* hort. ex I. Takah. | | | | Adults? (Beattie and Barkley 2009) | |
| | *Citrus decumana* L. | | | | Attacked (Beattie and Barkley 2009) | |
| *Citrus medica* L. | *Citrus medica* L. | | | Symptoms (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) | Preferred host (Beattie and Barkley 2009) |
| | *Citrus medica medica* | | | | Attacked (Beattie and Barkley 2009) | |
| *Citrus reticulata* Blanco | | Symptoms (Beattie and Barkley 2009) | Symptoms (Lopes and Frare 2008) | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) |
| | *Citrus deliciosa* Tenore | | | | Host (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) |
| | *Citrus depressa* Hayata | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Citrus keraji* Hort. ex Tanaka | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus nobilis* Lourerio | | | | Host (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) |
| | *Citrus nobilis* Loureiro var *deliclosa* (Ten.) Swingle | | | | Host (Beattie and Barkley 2009) | |
| | *Citrus oto* Hort. ex Yu.Tanaka | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus suhuiensis* Hort. ex Tanaka) | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus sunki* Hort. ex Tanaka | | Symptoms (Lopes and Frare 2008) | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus unshiu* (Mack.) | | | Symptoms (Beattie and | | |

| **Host Species** | **Host name as cited by the original author** | **'*Candidatus* Liberibacter species'** | | | **Psyllid vector** | |
|---|---|---|---|---|---|---|
| | | **Africanus** | **Americanus** | **Asiaticus** | ***Diaphorina citri*** | ***Trioza erytreae*** |
| | Marc) | | | Barkley 2009) | | |
| | *Citrus* species (mandarins and mandarin/sweet orange hybrids) | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus reticulata* x *Citrus sinensis* | | Symptoms (Lopes and Frare 2008) | | | |
| *Citrus reticulata* Blanco? | *Citrus reshni* | | Symptoms (Lopes and Frare 2008) | | | |
| *Citrus trifoliata* L. | *Poncirus trifoliata* (L.) Raf. | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | Eggs and adults, survival for seven weeks (Beattie and Barkley 2009) | Feeding (Beattie and Barkley 2009) |
| | Trifoliate orange | Symptoms (Beattie and Barkley 2009) | | | | |
| | Trifoliate orange and its hybrids | | | Symptoms (Beattie and Barkley 2009) | | |
| *Citrus trifoliata* L. x *Citrus paradisi*? | *Poncirus trifoliata x C. paradisi* | | Symptoms (Lopes and Frare 2008) | | | |
| *Citrus wintersii* Mabb. | *Microcitrus papauna* H. F. Winters | | | | Nymphs and adults (Beattie and Barkley 2009) | |
| *Citrus* x *aurantifolia* (Christm.) Swingle | *Citrus aurantifolia* (Christm.) Swingle | Symptoms (Beattie and Barkley 2009) | Symptoms (Lopes and Frare 2008) | Symptoms (Beattie and Barkley 2009) | Preferred host (Beattie and Barkley 2009) | Preferred host (Beattie and Barkley 2009) |
| | Limes | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus limmetioides* Tanaka | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Citrus pennivesiculata* (Lush.) Tanaka | | | | Moderate damage (Beattie and Barkley 2009) | |

| **Host Species** | **Host name as cited by the original author** | **'*Candidatus* Liberibacter species'** | | | **Psyllid vector** | |
|---|---|---|---|---|---|---|
| | | **Africanus** | **Americanus** | **Asiaticus** | ***Diaphorina citri*** | ***Trioza erytreae*** |
| *Citrus* x *aurantium* L. | *Citrus aurantium* L. | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | Eggs, nymphs and adults (Beattie and Barkley 2009) | |
| | Grapefruit, sweet orange and sour orange | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus hassaku* Hort. ex Tanaka | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus karna* Raf | | | Symptoms? (Beattie and Barkley 2009) | | |
| | *Citrus maderaspatana* Hort ex. Tanaka | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus maxima* var *racemosa* | | | | Host (Beattie and Barkley 2009) | |
| | Various cultivars | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus natsudaidai* Hayat) | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus nobilis* Lour. | | | Symptoms (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) | |
| | *Citrus x nobilis* | | | Host (FDA 2008) | Common (Beattie and Barkley 2009) | |
| | *Citrus paradisi* Macfadyen | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | | Common host (Beattie and Barkley 2009) |
| | *Citrus* x *paradisi* Macfadyen | | | Host (FDA 2008) | Common host (Beattie and Barkley 2009) | |
| | Minneola tangelo | Symptoms (Beattie and Barkley 2009) | | | | |
| | Orlando tangelo | Symptoms (Beattie | | | | |

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| | | and Barkley 2009) | | | | |
| | Yalaha and Orlando tangeloes | | | Severe symptoms (Beattie and Barkley 2009) | | |
| | *Citrus sinensis* (L.) Osbeck | Severe symptoms (Beattie and Barkley 2009) | Symptoms (Lopes and Frare 2008) | Severe symptoms (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) | Common host (Beattie and Barkley 2009) |
| | Grapefruit | Moderate symptoms (Beattie and Barkley 2009) | | Severe symptoms (Beattie and Barkley 2009) | | |
| | *Citrus sulcata* Hort. ex I. Takahashi | | | | Heavy psyllid damage (Beattie and Barkley 2009) | |
| | *Citrus tamurana* Hort. ex Tanaka | | | | Host (Beattie and Barkley 2009) | |
| *Citrus* x *insitorum* Mabb. | Troyer and Carrizo citrange | | | Symptomless to symptoms (Beattie and Barkley 2009) | | |
| | Troyer citrange | Symptoms (Beattie and Barkley 2009) | | | | |
| | X *Citroncirus webberi* J. Ingram & H. E. Moore | | | Few symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| *Citrus* x *junos* Siebold ex Tanaka; for '*Ca* L. species' | *Citrus junos* Siebold ex Tanaka | | | Symptoms (Beattie and Barkley 2009) | Some psyllid damage (Beattie and Barkley 2009) | |
| *Citrus* × *limon* (L.) Osbeck; for psyllids | *Citrus assamensis* S. Dutta and SC Bhattach | | | Symptoms (Beattie and Barkley 2009) | Good host (Beattie and Barkley 2009) | |
| | *Citrus limetta* (Risso) | | | Symptoms? (Beattie and Barkley 2009) | | |
| | *Citrus limon* (L.) Burman F | Moderate symptoms (Beattie | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | Preferred host (Beattie and |

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| | | and Barkley 2009) | | | | Barkley 2009) |
| | *Citrus limonia* Osbeck | | Symptoms (Lopes and Frare 2008) | Symptoms (Beattie and Barkley 2009) | Favoured host (Beattie and Barkley 2009) | |
| | *Citrus medica* var *acida* Hook. F. | | | | Attacked (Beattie and Barkley 2009) | |
| | *Citrus medica* var *limetta* | | | | Attacked (Beattie and Barkley 2009) | |
| | *Citrus medica* var *limonum* Hook. F. | | | | Attacked (Beattie and Barkley 2009) | |
| | *Citrus meyeri* Tanaka | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | Adults? (Beattie and Barkley 2009) | |
| | Lemons | | | Symptoms (Beattie and Barkley 2009) | | |
| *Citrus* x *latifolia* (Yu. Tanaka) Tanaka | *Citrus latifolia* (Yu. Tanaka) Tanaka | Symptoms (Beattie and Barkley 2009) | Symptoms (Lopes and Frare 2008) | Symptoms (Beattie and Barkley 2009) | | |
| *Citrus* x *microcarpa* Bunge | | | | Symptoms (Beattie and Barkley 2009) | | |
| | *Citrus madurensis* Lour. | | | | Host (Beattie and Barkley 2009) | |
| *Citrus* x *taitensis* Risso | *Citrus jambhiri* Lushington | Symptoms (Beattie and Barkley 2009) | | Symptoms (Beattie and Barkley 2009) | Some to moderate (Beattie and Barkley 2009) | |
| *Citrus* x *virgata* Mabb. | *Microcitrus* sp. 'Sydney' | | | | Damage, eggs and nymphs (Beattie and Barkley 2009) | |
| *Limonia acidissima* L. | | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| *Merrillia caloxylon* (Ridl.) Swingle | | | | | Lab (Beattie and Barkley 2009) | |
| *Murraya paniculata* (L.) Jack | *Murraya paniculata* | | Cryptic symptoms (Beattie and | Symptoms (Beattie and | Preferred host (Beattie and | Common host (Beattie and |

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| (*sensu lato*) | (L.) Jack | | Barkley 2009) | Barkley 2009) | Barkley 2009) | Barkley 2009) |
| | *Murraya paniculata* (L.) Jack var *paniculata* | | | No detection of HLB (Beattie and Barkley 2009) | | |
| | *Murraya paniculata* (L.) Jack var *jiulixiang* | | | No detection of HLB (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Murraya exotica* L. | | | Symptomless and non-persistent (Damsteegt *et al.* 2010). | Preferred host (Beattie and Barkley 2009) | Attacked slightly (Beattie and Barkley 2009) |
| *Naringi crenulata* (Roxb.) Nicolson | | | | | Heavy infestation (Beattie and Barkley 2009) | |
| *Pamburus missionis* (Wight) Swingle | *Pamburus missionis* (Wall. ex Wight) Oliver | | | | Eggs, nymphs and adults (Beattie and Barkley 2009) | |
| | *Atalantia missionis* Oliver | | | Symptoms (Beattie and Barkley 2009) | Feeding and transmission (Beattie and Barkley 2009) | |
| *Swinglea glutinosa* (Blanco) Merri. | *Swinglea glutinosa* | | | | Feeding in lab (Beattie and Barkley 2009) | |
| | *Swinglea glutinosa* (Blanco) Merr. | | | Symptoms (Beattie and Barkley 2009) | Damage, eggs and nymphs (Beattie and Barkley 2009) | |
| *Triphasia trifolia* (Burman f.) P. Wilson | *Triphasia trifolia* (Burman f.) P. Wilson | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Triphasia aurantiola Lour.* | | | | | Attacked slightly (Beattie and Barkley 2009) |
| **Rutaceae [Aurantioideae: Clauseneae]** | | | | | | |

| Host Species | Host name as cited by the original author | '*Candidatus* Liberibacter species' | | | Psyllid vector | |
|---|---|---|---|---|---|---|
| | | Africanus | Americanus | Asiaticus | *Diaphorina citri* | *Trioza erytreae* |
| *Murraya koenigii* L. | *Murraya euchrestrifolia* Hayata | | | No detection of HLB (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | *Murraya koenigii* (L.) Spreng. | | | Host (FDA 2008) | Host (Beattie and Barkley 2009) | |
| | *Bergera koenigii* L. | | | Not a host (Damsteegt *et al.* 2010) | Host (Damsteegt *et al.* 2010) | |
| *Clausena anisata* (Willd.) Benth. | *Clausena inaequalis* (DC) | | | Symptoms (Beattie and Barkley 2009) | | |
| *Clausena anisata* (Willd.)Hook. f. ex Benth. | *Clausena anisata* (Willd.)Hook. f. ex Benth. | | | | | Host (Beattie and Barkley 2009) |
| *Clausena anisumolens* (Blanco) Merr. | *Clausena anisum-olens* (Blanco) Merr. | | | | Feeding (Beattie and Barkley 2009) | |
| *Clausena excavata* Burm. f. | | | | | Host (Beattie and Barkley 2009) | |
| *Clausena indica* Oliver | *Clausena indica* (Dalz.) Oliver | | | Symptoms (Beattie and Barkley 2009) | Feeding and transmission (Beattie and Barkley 2009) | |
| *Clausena lansium* (Lour.) Skeels | | | | Symptoms (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) | |
| | | | | | | |
| **Rutaceae [Aurantioideae: Rutoideae]** | | | | | | |
| *Calodendrum capensis* (L. f) Thunb | *Calodendrum capensis* | Symptoms (Beattie and Barkley 2009)[††] | | | | Feeding (Beattie and Barkley 2009) |
| *Casimiroa edulis* La Llave & Lex. | | | | | | Feeding (Beattie and Barkley 2009) |
| *Ravenia spectabilis* (Lindl.) Planch. ex Griseb. | | | | | Adults? (Beattie and Barkley 2009) | |

| **Host Species** | **Host name as cited by the original author** | **'*Candidatus* Liberibacter species'** | | | **Psyllid vector** | |
|---|---|---|---|---|---|---|
| | | **Africanus** | **Americanus** | **Asiaticus** | ***Diaphorina citri*** | ***Trioza erytreae*** |
| *Tetradium ruticarpum* (A. Juss.) T. G. Hartley | *Evodia rutaecarpa* (A. Juss.) benth. | | | | Adults? (Beattie and Barkley 2009) | |
| *Toddalia asiatica* (L.) Lamarck | *Toddalia asiatica* | | | | Adult feeding (Beattie and Barkley 2009) | Feeding and oviposition (Beattie and Barkley 2009) |
| *Vepris lanceolata* (Lam.) G. Don | | Symptoms? (Beattie and Barkley 2009) | | | Adult feeding (Beattie and Barkley 2009) | Preferred host (Beattie and Barkley 2009) |
| | *Toddalia lanceolata* Lam | | | Host (FDA 2008) | | Preferred host (Beattie and Barkley 2009) |
| | *Vepris undulada* (Thunb.) Verdon & Smith | | | | | Preferred host (Beattie and Barkley 2009) |
| *Zanthoxylum capense* (Thunb.) Harv. | *Fagara capense* | | | | A few psyllids (Beattie and Barkley 2009) | Host (Beattie and Barkley 2009) |
| | *Fagara capense* Thunb. | | | | | Host (Beattie and Barkley 2009) |
| **Non–Rutaceae (experimental hosts)** | | | | | | |
| *Catharanthus roseus* (L.) G. Don [Gentianales: Apocynaceae] | *Vinca rosea* L. | | | Symptoms (Zhang *et al*. 2010) | | |
| *Cuscuta* species [Solanales: Convolvulaceae] | Cuscuta sp. (possibly *Cuscuta australis* R.Br.) | | | Symptoms (Beattie and Barkley 2009) | | |
| *Cuscuta australis* R. Br. [Solanales: Convolvulaceae] | | | | Symptomless? (Beattie and Barkley 2009) | | |
| | *Cuscuta campestris* Yuncker | | | Symptomless? (Beattie and Barkley 2009) | | |
| | *Cuscuta pentagona* Engelm. | | | Symptomless (Beattie and Barkley 2009) | | |

| **Host Species** | **Host name as cited by the original author** | **'*Candidatus* Liberibacter species'** | | | **Psyllid vector** | |
|---|---|---|---|---|---|---|
| | | **Africanus** | **Americanus** | **Asiaticus** | ***Diaphorina citri*** | ***Trioza erytreae*** |
| *Nicotiana tabacum* L. [Solanales: Solanaceae] | *Nicotiana xanthi* | | | Symptomless? (Beattie and Barkley 2009) | | |
| | *Nicotiana tabacum* cv. *xanthi* | | Symptoms (Beattie and Barkley 2009) | | | |
| *Solanum lycopersicum* L. [Solanales: Solanaceae] | *Lycopersicum esculentum* cvs Manapal, FL 47 | | | Symptoms (Beattie and Barkley 2009) | | |

† Host table of '*Ca* L. species' and psyllid vectors based on Beattie and Barkley (2009) with additional references as cited. Host taxonomy follows the convention of Beattie and Barkley (2009), host names as cited by the original author are presented in an additional column. Where host taxonomy is unclear from the additional references, this is reflected in the host species column with a question mark.
†† '*Ca*. L. africanus subsp. capensis'

# Glossary

| Term | Definition |
|---|---|
| **Additional declaration** | A statement that is required by an importing country to be entered on a phytosanitary certificate and which provides specific additional information pertinent to the phytosanitary condition of a consignment in relation to regulated pests (FAO 2007b). |
| **Appropriate level of protection** | The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1995). |
| **Area** | An officially defined country, part of a country or all or parts of several countries (FAO 2007b). |
| **Biosecurity Australia** | The unit within the Biosecurity Services Group responsible for recommendations for the development of Australia's biosecurity policy. |
| **Biosecurity Services Group** | The group responsible for the delivery of biosecurity policy and quarantine services within the Department of Agriculture, Fisheries and Forestry |
| **Consignment** | A quantity of plants, plant products and/or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more commodities or lots) (FAO 2007b). |
| **Control (of a pest)** | Suppression, containment or eradication of a pest population (FAO 2007b). |
| **Endangered area** | An area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss (FAO 2007b). |
| **Entry (of a pest)** | Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO 2007b). |
| **Establishment** | Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2007b). |
| **Establishment potential** | Likelihood of the establishment of a pest. |
| **Fresh** | Living; not dried, deep-frozen or otherwise conserved (FAO 2007b). |
| **Fruits and vegetables** | A commodity class for fresh parts of plants intended for consumption or processing and not for planting (FAO 2007b). |
| **Host** | A species of plant capable, under natural conditions, of sustaining a specific pest. |
| **Import Permit** | Official document authorising importation of a commodity in accordance with specified phytosanitary import requirements (FAO 2007b). |
| **Infestation (of a commodity)** | Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (FAO 2007b). |
| **Inspection** | Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations (FAO 2007b). |
| **Intended use** | Declared purpose for which plants, plant products, or other regulated articles are imported, produced, or used (FAO 2007b). |
| **Interception (of a pest)** | The detection of a pest during inspection or testing of an imported consignment (FAO 2007b). |
| **Introduction** | The entry of a pest resulting in its establishment (FAO 2007b). |
| **Lot** | A number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (FAO 2007b). |
| **National Plant Protection Organisation** | Official service established by a government to discharge the functions specified by the IPPC (FAO 2007b). DAFF is Australia's National Plant Protection Organisation. |
| **Official control** | The active enforcement of mandatory phytosanitary regulations and the application of mandatory phytosanitary procedures with the objective of eradication or containment of quarantine pests or for the management of regulated non-quarantine pests (FAO 2007b). |
| **Pathway** | Any means that allows the entry or spread of a pest (FAO 2007b). |
| **Pest** | Any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or |

| Term | Definition |
|---|---|
| | plant products (FAO 2007b). |
| **Pest categorisation** | The process for determining whether a pest has or has not the characteristics of a quarantine pest or those of a regulated non-quarantine pest (FAO 2007b). |
| **Pest free area** | An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2007b). |
| **Pest risk analysis** | The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated and the strength of any phytosanitary measures to be taken against it (FAO 2007b). |
| **Pest risk assessment (for quarantine pests)** | Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (FAO 2007b). |
| **Pest risk management (for quarantine pests)** | Evaluation and selection of options to reduce the risk of introduction and spread of a pest (FAO 2007b). |
| **Phytosanitary certificate** | Certificate patterned after the model certificates of the IPPC (FAO 2007b). |
| **Phytosanitary measure** | Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO 2007b). |
| **Phytosanitary regulation** | Official rule to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests, including establishment of procedures for phytosanitary certification (FAO 2007b). |
| **Polymerase chain reaction** | A technique that utilises a heat stable DNA polymerase to amplify a piece of DNA by *in vitro* enzymatic replication, initiating a chain reaction in which the DNA template is exponentially amplified, generating millions or more copies of the target DNA. |
| **Polyphagous** | Feeding on a relatively large number of host plants from different plant families. |
| **Protected area** | A regulated area that an NPPO has determined to be the minimum area necessary for the effective protection of an endangered area (FAO 2007b). |
| **Quarantine pest** | A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2007b). |
| **Regulated article** | Any plant, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material capable of harbouring or spreading pests, deemed to require phytosanitary measures, particularly where international transportation is involved (FAO 2007b). |
| **Restricted risk** | 'Restricted' risk estimates apply to situations where risk management measures are used |
| **Spread** | Expansion of the geographical distribution of a pest within an area (FAO 2007b). |
| **SPS Agreement** | WTO Agreement on the Application of Sanitary and Phytosanitary Measures (WTO 1995). |
| **Stakeholders** | Government agencies, individuals, community or industry groups or organisations, whether in Australia or overseas, including the proponent/applicant for a specific proposal. |
| **Systems approach(es)** | The integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of phytosanitary protection (FAO 2007b). |
| **Unrestricted risk** | 'Unrestricted' risk estimates apply in the absence of risk management measures. |

## References

Ahmed S, Ahmad N, Khan RR (2004) Studies on population dynamics and chemical control of citrus psylla, *Diaphorina citri*. *International Journal of Agriculture and Biology* 6: 970–973.

Albrecht U, Bowman KD (2008) Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus* Liberibacter asiaticus causing Huanglongbing in Florida. *Plant Science* 175: 291–306.

APHIS (2008a) Federal Domestic Quarantine Order: Citrus Greening Disease and Asian Citrus Psyllid, June 24, 2008.

APHIS (2008b) Federal Import Quarantine Order for Huanglongbing (HLB) disease of Citrus and Citrus Variegated Chlorosis (CVC); DA-2008-06; January 29, 2008. Animal and Plant Health Inspection Service, USDA.

APNI (2008) Australian Plant Name Index. Integrated Botanical Information System (IBIS), Australian National Botanic Gardens, Australian National Herbarium. http://www.anbg.gov.au/cpbr/databases/apni-search-names-only.html (Accessed 19 June 2009).

Associated Press (2009) HLB positive Asian citrus psyllid found in California. http://www.cloudforest.com/cafe/forum/66269.html Accessed 15/9/2009.

Atwal AS, Chaudhary JP, Ramzan M (1970) Studies on the development and field population of citrus psylla, *Diaphoprina citri* Kuwayama (Psyllidae: Homoptera). *Journal of Research, Punjab Agriculture University* 7: 333–338.

Aubert B (1987a) Epidemiological aspects of the greening (huanglongbing) disease in Asia. In: *The FAO-UNDP Project Coordinator in Regional Workshop on Citrus Greening Huanglongbing Disease*, (ed Fuzhou ) 5 pp., Fuzhou, China, December 1987.

Aubert B (1987b) *Trioza erytreae* (Del Guercio) and *Diaphorina citri* Kuwana (Homoptera: Psylloidea), the two vectors of citrus greening disease: biological aspects and possible control strategies. *Fruits* 42: 149–162.

Aubert B (1988) Management of the citrus greening disease in Asian orchards. In: *Proceedings of the Second Asian/Pacific Regional Workshop on citrus greening* (eds Aubert B, Ke C, Gonzales C) pp. 51–52 Lipa, Philippines, November 1988. Rome: UNDP-FAO.

Aubert B (1990) Integrated activities for the control of huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia. In: *Proceedings of the Fourth International Asia Pacific Conference on Citrus Rehabilitation* (eds Aubert B, Tontyaporn S, Buangsuwon D) pp. 133–144. Chiang Mai, Thailand, February 1990. Rome: FAO UNDP.

Aubert B (2008) Historical perspectives of HLB in Asia. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 16–24, Orlando, Florida 2008. www.plantmanagementnetwork.org

Aubert B, Bové T J M, Etienne J (1980) La lutte contre la maladie du 'greening' des agrumes á l'Ile de la RTunion. RTsultats et perspectives. *Fruits* 35: 605–624.

Aubert B, Garnier M, Guillaumin D, Herbagyandono B, Setiobudi L, Nurhadi F (1985) Greening, a serious threat for the citrus productions of the Indonesian Archipelago. Future prospects of integrated control. *Fruits* 40: 549–563.

Aubert B, Quilici S (1984) Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Réunion Island. In: *Proceedings of the 9th Conference of the International of Citrus Virologists*. (eds Garnsey SM, Timmer LW, Dodds JA) pp.100–108. University of California, Riverside, USA: IOCV.

Aubert B, Xia YH (1990) Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies. In: *Proceedings of the Fourth International Asia Pacific Conference on Citrus Rehabilitation* (eds Aubert B, Tontyaporn S, Buangsuwon D) pp. 181–187, Chiang Mai, Thailand, February 1990. Rome: FAO UNDP.

Barkley PB, Beattie GAC (2008) Contingency plans for HLB (Huanglongbing) and its vectors in Australia. In: *I Taller Internacional sobre Huanglongbing de los críticos* ('Candidatus *Liberibacter* spp.') *y el psílido asiático de los críticos* (Diaphorina citri). Hermosillo, Sonora. Mexico.

Barton LV (1943) The storage of citrus seed. *Contributions from Boyce Thompson Institute* 13: 1–47.

Bastianel C, Garnier-Semancik M, Renaudin J, Bové JM, Eveillard S (2005) Diversity of '*Candidatus* Liberibacter asiaticus', based on the *omp* gene sequence. *Applied and Environmental Microbiology* 71: 6473–6478.

Bayer RS, Mabberley DJ, Morton C, Miller C, Sharma I, Pfeil B, Rich S, Hitchcock R, Sykes S (2009) A molecular phylogeny of the orange subfamily (Rutaceae: Aurantioideae) using nine cpDNA sequences. *American Journal of Botany* 96: 668–685.

Beattie GAC, Barkley, P (2009) *Huanglongbing and its vectors. A Pest Specific Contingency Plan for the Citrus and Nursery and Garden Industries*, Version 2: 10 February 2009, 272 pp.

Beattie GAC, Holford P, Mabberley DJ, Haigh AM, Bayer R, Broadbent P. (2006) Aspects and insights of Australia-Asia collaborative research on huanglongbing. In: *Proceedings of an International Workshop for the Prevention of Citrus Greening Disease in Severely Infected Areas* pp. 47–64, Ishigaki, Japan, December 2006. Tokyo: Multilateral Research Network for Food and Agricultural Safety. Japanese Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan.

Beattie GAC, Holford P, Mabberley DJ, Haigh AM, Broadbent P (2008) On the origin of *Citrus*, Huanglongbing, *Diaphorina citri* and *Trioza erytreae*. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 23–56, Orlando, Florida 2008. www.plantmanagementnetwork.org

Bellis G, Hollis D, Jacobson S (2005) Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), do not exist in the Stapleton Station area of the Northern Territory of Australia. *Australian Journal of Entomology* 44**:** 68–70.

Benyon L, Zhou L, Daun Y, McCollum G, Powell C, Hall D, Irey M, Gottwald T (2009) Seed transmission of '*Candidatus* Liberibacter asiaticus' in citrus without typical huanglongbing. *Phytopathology* 99 (Supplement): S11.

Benyon LS, Zhou LJ, Powell CA, Gottwald T, Duan YP (2008) Improved detection of low-titer, non-lethal, seed transmitted '*Candidatus* Liberibacter *asiaticus'* in citrus, periwinkle and dodder using nested PCR. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 120–123, Orlando, Florida 2008. www.plantmanagementnetwork.org

Bonani JP, Appezzato-da-Gloria B, Fereres A, Engels FE, Lopes JRS (2008) Leaf age influencing acquisition of *Candidatus* Liberibacter asiaticus by the psyllid vector, *Diaphorina citri* Kuwayama. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 249, Orlando, Florida 2008. www.plantmanagementnetwork.org

Bové JM (2006) Huanlongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* 88: 7–37.

Bové JM, Ayres AJ (2007) Etiology of three recent diseases of Citrus in Sao Paulo State: Sudden death, Variegated chlorosis and Huanglongbing. *Life*: 59: 346–354.

Bové JM, Dwiastuti ME, Trivatno A, Supriyanto A, Nasli E, Becu P, Garnier M (2000) Incidence of huanglongbing and citrus rehabilitation in North Bali, Indonesia. In: *Proceedings of the Fourteenth Conference of the International Organization of Citrus Virologists* (eds da Graça JV, Lee RF, Yokomi RK) pp. 200–206, Campinas, Sao Paulo, Brazil, September 1998. Riverside: International Organization of Citrus Virologists, University of California: Riverside.

Bové JM, Garnier M, Ahlawat YS, Chakraborty NK, Varma A (1993) Detection of the Asian strains of the greening BLO by DNA hybridization in Indian orchard trees and Malaysian *Diaphorina citri* psyllids. In: *Proc. 12th Conference of the International Organization of Citrus Virologists* (eds Moreno P, da Graça JV, Timmer LW) pp. 258–263. University of California, Riverside.

Bové JM, Teixeira DC, Wulff NA, Eveillard S, Sailard C, Bassanezi RB, Lopes S, Yammamoto, PT, Ayers AJ (2008) Several Liberibacter and Phytoplasma Species are Individually Associated with HLB. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 152–155, Orlando, Florida 2008. www.plantmanagementnetwork.org

Bové, JM (1995) *Virus and virus-like diseases of citrus in the Near East region*. FAO, Rome, Italy. pp. 283–302.

Bray RA, Sands DPA (1987) Arrival of the leucaena psyllid in Australia: impact, dispersal and natural enemies. *Leucaena Research Reports* 7: 61–65.

Brlansky RH, Rogers ME (2007) Citrus Huanlongbing: understanding the vector-pathogen interaction for disease management. *APSnet feature, the American Phytopathological Society*. Online at: http://www.apsnet.org/online/feature/HLB/ (accessed 15 July 2009).

Broadbent P (1995) Quarantine in relation to Australian citrus imports and exports. *Australasian Plant Pathology* 24: 145–156.

Buitendag C H, von Broembsen LA (1993) Living with citrus greening disease. In: *Proceedings of the 12th Conference for the International Organisation of Citrus Virologists*. (eds. Moreno P, da Graça J V, Timmer L W) pp. 269–271. IOCV, Riverside, California, USA.

Bunn D, Feenstra GW, Lynch L, Summer R (1990). Consumer acceptance of cosmetically imperfect produce. *The Journal of Consumer Affairs* 24: 268–279.

Burckhardt D (1984) The Mediterranean species of *Diaphorina* Loew (Homoptera, Psylloidea). *Phytophaga* 2: 1–30.

Burckhardt D (1994) Psylloid pests of temperate and subtropical crop and ornamental plants (Hemiptera, Psylloidea): a review. *Trends Agricultural Science Entomology* 2: 173–186.

CABI (2007) Crop Protection Compendium. CAB International, Wallingford, UK. http://www.cabicompendium.org/cpc/home.asp (Accessed 15 June 2009).

CABI/EPPO (2006) *Trioza erytreae distribution maps of plant pests No. 234*. CABI International, Wallingford, UK.

Capoor SP, Rao DG, Viswanath SM (1974) Greening disease of citrus in the Deccan Trap country and its relationship with the vector *Diaphorina citri* Kuwayama. In: *Proceedings of the Sixth Conference of the International Organization of Citrus Virologists* (eds Weathers LG, Cohen M) pp. 43–49. Mbabane, Swaziland, August 1972. Richmond: University of California, Division of Agricultural Sciences.

Catling HD (1969a) The bionomics of the South African psylla, *Trioza erytrae* D. G. (Homoptera:Psyllidae) 3. The influence of extremes of weather on survival. *Journal of the Entomological Society of South Africa* 32: 273–290.

Catling HD (1969b) The control of citrus psylla *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). *South African Citrus Journal* 426: 9–16.

Catling HD (1970) Distribution of the psyllid vectors of citrus greening disease with notes on the biology and bionomics of *Diaphorina citri*. *FAO Plant Protection Bulletin* 18: 8–15.

Catling HD (1971) The bionomics of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae) 5. The influence of host plant quality. *Journal of the Entomological Society of Southern Africa* 34: 381–391.

Catling HD (1972) Notes on the biology of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). *Journal of the Entomological Society of Southern Africa* 36: 299–306.

Catling HD, Atkinson PR (1974) Spread of greening by *Trioza erytreae* (Del Guercio) in Swaziland. In: *Proceedings of the Sixth Conference of the International Organization* *of Citrus Virologists* (eds Weathers LG, Cohen M) pp. 33–39, Mbabane, Swaziland, August 1972. Richmond: University of California, Division of Agricultural Sciences.

Catling, HD (1973) Notes on the biology of the South African citrus psylla *Trioza erytreae*. *Journal of the Entomological Society of South Africa* 36: 299–306.

CDFA (2008a) Asian Citrus Psyllid Pest Profile. California Department of Food and Agriculture, 1220 N Street Sacramento, California http://www.cdfa.ca.gov/phpps/PDEP/target_pest_disease_profiles/ACP_PestProfile.html. (Accessed 5 February 2009).

CDFA (2008b) Country High Risk Program–Examples of High Risk Inspections in 2008. Plant Health and Pest Prevention Services, Californian Department of Food and Agriculture. http://www.cdfa.ca.gov./phpps/ar/pe_interior_chr2.html (Accessed 17 August 2009).

CDFA (2009) Survey for Asian Citrus Psyllids in Fresno Tomorrow. News release #09–058, California department of Agriculture. http://www.cdfa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=09-058 (Accessed 17 August 2009).

Cermeli M, Morales P, Godoy F (2000) Presence of the Asiatic citrus psyllid, *Diaphorina citri* Kuwayarna (Hemiptera: Psyllidae) in Venezuela. *Boletin de Entomologia Venezolana* 15: 235–243.

Chakravarthi VP, Savithri P, Prasad PR, Naidu VG (1998) Efficacy of various insecticides against citrus psylla, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). In: *Proceedings of the First National Symposium on Pest Management in Horticultural Crops: Environmental Implications and Thrusts* (eds. Reddy PP, Kumar NKK, Verghese A) pp. 32–33, Bangalore, India.

Chavan VM (2004) Investigations on citrus psylla, *Diaphorina citri* Ku., vector of citrus greening disease in Maharashtra. *Journal of Maharashtra Agricultural University* 29: 290–293.

Cheema SS, Chohan JS, Kapur SP (1982) Effect of moist hot air on citrus greening infected budwood. *Journal of Research, India* 19: 97–99.

Codex (2005) Codex Standard for Oranges (CODEX STAN 245–2004, AMD. 1-2005) *Codex Alimentarius*. http://www.codexalimentarius.net/web/index_en.jsp# (Accessed 23 July 2009)

Coletta-Filho HD, Carlos EF, Alves KCS, Pereira MAR, Boscariol-Camargo RL, de Souza AA, Machado MA (2010) In planta multiplication and grat transmission of '*Candidatus* Liberibacter asiaticus' revealed by Real-Time PCR. *European Journal of Plant Pathology* 126: 53–60.

Conant P, Hirayana C, Kumashiro BR, Heu RA, Young CL (2009) New Pest Advisory: Asian Citrus psyllid. State of Hawaii Department of Agriculture. http://hawaii.gov/hdoa/pi/ppc/npa-1/npa06-01-ACP.pdf

da Graça JV (1991) Citrus greening disease. *Annual Review of Phytopathology* 29: 109–136.

da Graça JV (2008) Biology, history and world status of Huanglongbing. In: *I Taller Internacional sobre Huanglongbing de los críticos* ('Candidatus *Liberibacter* spp.') *y el psílido asiático de los críticos* (Diaphorina citri). Hermosillo, Sonora. Mexico.

Dai Y, Chen N, Chen X, Liao C, Cai M, Shen J, Chen Z (1982) Studies on the vector of citrus huanglongbing citrus psylla *Diaphorina citr*i Kuw. *China Citrus* 3: 1–2 (in Chinese).

Damsteegt VD, Postnikova EN, Stone AL, Kuhlmann M, Wilson C, Sechler A, Schaad NW, Brlansky RH, Schneider WL (2010) *Murraya paniculata* and related species as potential hosts and inoculum reservoirs of '*Candidatus* Liberibacter asiaticus', causal agent of Huanglongbing. *Plant Disease* 94: 528–533.

Das AK, Kumar A (2010) First report of the Huanglongbing bacterium '*Candidatus* Liberibacter asiaticus' infecting Satkara (*Citrus macroptera*) in India. *Plant Disease* 94: 375.

Davies FS, Albrigo LG (1994) *Citrus*. Cab International, Wallingford Oxon OX10 8DE, UK

DEWHA (2009) EPBC Act List of Threatened Flora. Department of Environment, Heritage, Water and Arts. http://www.environment.gov.au/cgi-bin/sprat/public/publicthreatenedlist.pl?wanted=flora (Accessed 15 July 2009).

Doddapaneni H, Liao HH, Lin H, Bai XJ, Zhao XL, Civerolo EL, Irey M, Coletta-Filho H, Pietersen G (2008) Comparative phylogenomics and multi-gene analyses of the citrus huanglongbing (HLB)-associated bacterium *Candidatus* Liberibacter. *BMC Research Notes* 1: 72. 22 p. doi:10.1186/1756-0500-1-72.

Duan YP, Gottwald T, Zhou LJ, Gabriel DW (2008) First report of Dodder transmission of *Candidatus* Liberibacter asiaticus to tomato (*Lycopersicon esculentum*). *Plant Disease* 92: 831.

Edwards CM, Mumford PM (1985) The effect of drying methods on the behaviour of sour orange (*Citrus aurantium*) seeds. *Seed Science and Technology* 13: 227–234.

EPPO (2005a) *Trioza erytreae. OEPP/EPPO Bulletin* 35: 357–360.

EPPO (2005b) *Diaphorina citri. OEPP/EPPO Bulletin* 35: 331–333.

EPPO (2009) Data Sheets on Quarantine Pests: *Diaphorina citri*. Prepared by CABI and EPPO for the EU under Contract 90/399003. http://www.eppo.org/QUARANTINE/insects/Diaphorina_citri/DIAACI_ds.pdf (Accessed 10 December 2009).

Evers G, Grisoni M (1991) Present situation of the citrus greening disease in Tanzania and proposal for control strategies. *Fruits* 46: 171–177.

Examiner (2009) Citrus pest discovered in Somerton, state quarantine expected. http://www.examiner.com/x-4805-Arizona-Rural-Headlines-Examiner~y2009m11d4-Citrus-pest-discovered-in-Somerton-state-quarantine-expected (Accessed 5 November 2009).

Faghihi MM, Salehi M, Bagheri A, Izadpanah K (2009) First report of citrus huanglongbing disease on orange in Iran. *Plant Pathology* 58: 793.

FAO (1996) Establishment of pest free areas. *International Standards for Phytosanitary Measures*, No 04. International Plant Protection Convention, Food and Agriculture Organization of the United Nations.

FAO (1999) Requirements for the establishment of pest free places of production and pest free production sites. *International Standards for Phytosanitary Measures*, No 10. International Plant Protection Convention, Food and Agriculture Organization of the United Nations.

FAO (2004) ISPM 11: Pest Risk Analysis for Quarantine Pests, including analysis of environmental risks and living modified organisms. *International Standards for Phytosanitary Measures*, No 11. International Plant Protection Convention, Food and Agriculture Organization of the United Nations.

FAO (2007) Framework for Pest Risk Analysis. *International Standards for Phytosanitary Measures*, No 02. International Plant Protection Convention, Food and Agriculture Organization of the United Nations.

FAO (2009) Glossary of Phytosanitary terms. *International Standards for Phytosanitary Measures*, No 05. International Plant Protection Convention, Food and Agriculture Organization of the United Nations.

FDA (2008) Known Host plants of Huanglongbing (HLB) and Asian Citrus Psyllid. Florida Department of Agriculture, Division of Plant Industry. http://www.doacs.state.fl.us/pi/chrp/greening/hostlist.pdf (Accessed 7 July 2009)

Filippini K (2008) Pest exclusion advisory 03-2008. Sacramento: State of California Department of Food and Agriculture.

Floyd J, Krass C (2006) New Pest Response Guidelines: Huanglongbing Disease of Citrus.USDA/APHIS/PPQ-Emergency and Domestic Programs, Riverdale, Maryland. http://www.aphis.usda.gov/import_export/plants/ppq_manuals.shtml (Accessed 16 June 2009).

Folimonova SY, Robertson CJ, Garnsey SM, Gowda S, Dawson WO (2009) Examination of the responses of different genotypes of citrus to Huanglongbing (citrus greening) under different conditions. *Phytopathology* 99: 1346–1354.

Francischini FJB, Oliveira KDS, Astua-Monge G, Novelli A, Lorenzino R, Matiolli C, Kemper E, da Silva ACR (2007) First report on the transmission of *'Candidatus* Liberibacter americanus' from citrus to *Nicotiana tabacum* cv. Xanthi. *Plant Disease* 91: 631.

Futch S, Brlansky R, Irey M, Weingarten S (2008) Detection of *Candidatus* Liberibacter asiaticus in sprouts from citrus tree stumps. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 353–354, Orlando, Florida 2008. www.plantmanagementnetwork.org

Garnier M, Bové JM (1983) Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. *Phytopathology* 73: 1358–1363.

Garnier M, Bové JM. (1993) Citrus greening disease and the greening bacterium. In: *Proceedings of the Twelfth Conference of the International Organization of Citrus Virologists* (eds Moreno P, da Graça JV, Timmer LW) pp. 212–219, New Delhi, India, November 1992. Riverside: International Organization of Citrus Virologists, University of California, Riverside.

Garnier M, Gao SJ, He Y, Villechanoux S, Gandar J, Bové JM (1991) Study of the greening organism (GO) with monoclonal antibodies: serological identification, morphology serotypes and purification of the GO. In: *Proceedings of the Eleventh Conference of the International Organization of Citrus Virologists* (eds Brlansky RH, Lee RF, Timmer LW) pp. 428–435. Orlando, Florida, November 1989. Riverside: International Organization of Citrus Virologists, University of California: Riverside.

Gasparoto MCG, Bassanezi RB, Lopes SA, Frare G, Martins EC, Della Coletta-Filho H, Amorim L (2008) Influence of temperature on Huanglongbing infection under controlled environment. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 186–187, Orlando, Florida 2008. www.plantmanagementnetwork.org

Ghosh SC, Giannotti J, Louis C (1977) Multiplication intensive des prokaryotes associés aux maladies de type "greening" des agrumes dans les cellules criblées de cuscute. *Annals of Phytopathology* 9: 525–530.

Gonzales CI, Viñas RC (1981) Field performance of citrus varieties and cultivars grown under control measures adopted against leaf mottling (greening) disease in the Philippines. In: *Proceedings of the Fourth International Society of Citriculture Congress* (ed Matsumoto K) pp. 463–464, Tokyo, Japan, November 1981. Riverside: International Society of Citriculture.

Gottwald TR, Aubert B, Xue-Yuan Z (1989) Preliminary analysis of citrus greening (Huanglungbin) epidemics in the People's Republic of China and French Réunion Island. *Phytopathology* 79: 687–693.

Gottwald TR, da Graça JV, Bassanezi RB (2007) Citrus Huanglongbing: the pathogen and its impact. Plant Health Progress, Plant Management Network publication.

Grafton-Cardwell EE, Godfrey KE, Rogers ME, Childers CC, Stansly PA (2006) Publication 8205. University of California, Division of Agriculture and Natural Resources, Exotic and Invasive Pests and Diseases Research Program.

Graham JH, Irey MS, Dawson WO, Hall D, Duan Y (2008) Assessment of transmission of Liberibacter asiaticus from seed to seedlings of 'Pineapple' sweet orange and Carrizo citrange. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 199–200, Orlando, Florida 2008. www.plantmanagementnetwork.org

Green GC, Catling HD (1971) Weather-induced mortality of the citrus psylla, *Trioza erytreae* (Del Guárico) (Homoptera: Psyllidae), a vector of greening virus, in some citrus producing areas of Southern Africa. *Agricultural Meteorology* 8: 305–317.

Halbert SE, Manjunath KL (2004) Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. *Florida Entomologist* 87: 330–353.

Halbert SE, Manjunath KL, Brodie MW (2008) Large-scale distribution of *Diaphorina citri* Kuwayama and citrus Huanglongbing in Florida. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 112–115, Orlando, Florida 2008. www.plantmanagementnetwork.org

Halbert SE, Niblett CL, Manjunath KL, Lee RF (2002) Establishment of two new vectors of citrus pathogens in Florida. In: *Proc. International Soc. Citriculture IX Congress* pp 1016–1017 ASHS Press, Alexandria, VA.

Halbert SE, Núñez CA (2004) Distribution of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Rhynchota: Psyllidae) in the Caribbean basin. *Florida Entomologist* 87: 401–402.

Halbert SE, Manjunath KL, Ramadugu C, Brodie MW, Webb SE, Lee RF (2010) Trailers transporting oranges to processing plants move Asian citrus psyllids. *Florida Entomologist* 93: 33–38.

Hall DG (2008a) Biology, history and world status of *Diaphorina citri*. In: *I Taller Internacional sobre Huanglongbing de los críticos* ('Candidatus *Liberibacter* spp.') *y el psílido asiático de los críticos* (Diaphorina citri). Hermosillo, Sonora Mexico.

Hall DG (2008b) Effects of freezes on survival of *Diaphorina citri*. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 219–221, Orlando, Florida 2008. www.plantmanagementnetwork.org

Hartung JS, Halbert S, Shatters R (2008) Can *Ca* Liberibacter asiaticus be transmitted through seed. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 188, Orlando, Florida 2008. www.plantmanagementnetwork.org

Hollis D (1984) Afrotropical jumping plant lice of the family Triozidae (Homoptera: Psylloidea). *Bulletin of the British Museum (Natural History), Ent*omology 49: 1–102.

Hollis D (1987) A new citrus-feeding psyllid from the Comoro Islands, with a review of the *Diaphorina amoena* species group (Homoptera). *Systematic Entomology* 12: 47–61.

Huang B (1990) Citrus psyllid. Chinese Agricultural Encyclopedia (Volume Insects). Beijing: Agriculture Press. pp 119–120.

Huang CH (1978) Effect of hot-air treatment on likubin, tristeza virus and exocortis viroid. *Journal of Agricultural Research of China* 27: 193–197.

Huang CH (1979) Distribution of likubin pathogen in likubin-affected plants. *Journal of Agricultural Research of China* 28: 29–33.

Huang CH, Liaw CF, Chang L, Lan T (1990) Incidence and spread of citrus likubin in relation to the population fluctuation of *Diaphorina citri*. *Plant Protection Bulletin* (Taipei) 32: 167–176.

Huang J, Luo X, Huang B, Yao X (1999) Studies on citrus psylla *Diaphorina citri* Kuwayama and its control. *Entomological Journal of East China* 8: 26–34. (In Chinese with English abstract).

Hung TH, Wu ML, Su HJ (1999) Development of a rapid method for the diagnosis of citrus greening disease using the polymerase chain reaction. *Journal of Phytopathology* 147: 599.

Hung TH, Wu ML, Su HJ (2000) Identification of alternative hosts of the fastidious bacterium causing citrus greening disease. *Journal of Phytopathology* 148: 321–326.

Hung TH, Wu ML, Su HJ (2001) Identification of the Chinese box orange (*Severinia buxifolia*) as an alternative host of the bacterium causing citrus huanglongbing. *European Journal of Plant Pathology* 107: 183–189.

Hung, TH, Hung, SC, Chen, CN, Hsu, MH, Su, HJ (2004) Detection by PCR of *Candidatus* Liberibacter asiaticus, the bacterium causing citrus huanglongbing in vector psyllids: Application to the study of vector-pathogen relationships. *Plant Pathology* 53: 96–102.

Husain MA, Nath D (1927) The citrus psylla (Diaphorina citri Kuw.) [Psyllidae: Homoptera]. Memoirs of the Department of Agriculture India, Entomology Series 10: 5–27.

Inoue H, Shinohara K, Okumura M, Ikeda K, Ashihara W, Ohira Y (2006) Occurrence of *Psylla evodiae* Miyatake (Hemiptera: Psyllidae) on the cultivated orange jasmine, *Murraya paniculata* (Rutaceae) in Kyushu and on Yakushima Island. *Japanese Journal of Applied Entomology and Zoology* 50: 66–68

Irey MA, Gast T, Snively J (2008) Grove management at Southern Gardens citrus in the presence on Huanlongbing. In: *I Taller Internacional sobre Huanglongbing de los críticos* ('Candidatus *Liberibacter* spp.') *y el psílido asiático de los críticos* (Diaphorina citri). Hermosillo, Sonora. Mexico 2008.

Irey MS (2006) Sensitivity of PCR-based methods for detection of Asiatic strain of the huanglongbing bacterium in symptomatic and asymptomatic citrus tissues (Abstr.). pp. 19. In: *Proc. Huanglongbing-Greening International Workshop*. Riberao Preto, Brazil.

Irey MS, Gast T, Gottwald TR (2006) Comparison of visual assessment and polymerase chain reaction assay testing to estimate the incidence of the Huanglongbing pathogen in commercial Florida citrus. *Proceedings of Florida State Horticultural Society* 119: 89–93.

Jagoueix S, Bové JM, Garnier M (1996) PCR detection of the two '*Candidatus* Liberibacter species' associated with greening disease of citrus. *Molecular and cellular Probes* 10: 43–50.

Jagoueix S, Bové´ JM, Garnier M (1994) The phloem-limited bacterium of greening disease of citrus is a member of the subdivision of the Proteobacteria. *International Journal of Sysematic Bacteriology* 44: 379–386.

Ke S, Li KB, Ke C, Tsai JH (1988) Transmission of the Huanglungbing agent from citrus to periwinkle by dodder. In: *Proceedings of the 10th Conference of International Organization of Citrus Virologists* (eds Garnsey SM, Timmer LM) pp. 258–264. Riverside, California.

Kim JS, Sagaram US, Burns JK, Li JL, Wang N (2009) Response of sweet orange (*Citrus sinensis*) to '*Candidatus* Liberibacter asiaticus' infection: microscopy and microarray analysis. *Phytopathology* 99: 50–57.

Knapp JL, Halbert S, Lee R, Hoy M, Clark R, Kesinger M (2006) Asian citrus psyllid and citrus greening disease. University of Florida, Integrated Pest Management Program. Online at: http://ipm.ifas.edu/agricultrue/citrus/asian.shtml (Accessed 16 June 2009).

Lahiri AR, Biswas S (1980) Observations on the relative intensity of infection on three species of cultivated citrus plants by *Psylla murrayi* Mathur (Homoptera: Psyllidae) at Shillong, Meghalaya. *Bulletin of the Zoological Survey of India* 2: 123–127.

Lama TK, Regmi C, Aubert B (1987) Distribution of the Citrus Greening Disease Vector (*Diaphorina citri* Kuw.) in Nepal and Attempts to Establish Biological Control. *Proceedings of the 10th Conference of the International Organization of Citrus Virologists* pp. 255–257.

Le Roux HF, Van Vuuren, SP, Pretorius, MC, Buitendag, CH (2006) Management of huanglongbing in South Africa. In: *Proc. of the Huanglongbing-Greening Intl. Workshop*, pp. 43–47, Ribeirão, Brazil.

Li W, Hartung JS, Levy L (2007) Evaluation of DNA amplification methods for improved detection of "*Candidatus* Liberibacter species" associated with citrus Huanglongbing. *Plant Disease* 91: 51–58.

Li W, Levy L, Hartung JS (2009) Quantitative distribution of '*Candidatus* Liberibacter asiaticus' in citrus plants with citrus huanglongbing. *Phytopathology* 99: 139–144.

Li W, Li D, Twieg E, Hartung JS, Levy L (2008) Optimized quantification of unculturable *Candidatus* Liberibacter species in host plants using real-time PCR. *Plant Disease* 92: 854–861.

Lin LH, Lin LH (1990) The citrus huang lung bin (greening) disease in China. In: Proceedings of the Fourth International Asia Pacific Conference on Citrus Rehabilitation (eds Aubert B, Tontyaporn S, Buangsuwon D) pp. 1–26, Chiang Mai, Thailand, February 1990. Rome: FAO UNDP.

Lin SJ, Ke YF, Tao CC (1973) Bionomics, observation and integrated control of citrus psylla, *Diaphorina citri* Kuwayama. *Journal of the Horticultural Society of China* 19: 235–242.

Liu YH, Tsai JH (2000) Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). *Annals of Applied Biology*: 137: 201–206.

Llauger R, Luis M, Collazo C, Pena I, Gonzalez C, Batista L, Teixeira D, Matins E, Perdomo A, Casin JC, Perez JL, Cueto JR, Bové JM (2008) Current situation of citrus Huanglongbing in Cuba. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 99–102, Orlando, Florida 2008. www.plantmanagementnetwork.org

Lopes S (2009) Current HLB research in Brazil. *Phytopathology* 99 (Supplement): S156.

Lopes SA, Bertolini E, Frare GF, Martins EC, Wulff NA, Teixeira DC, Fernandes NG, Cambra M (2009b) Graft transmission efficiencies and multiplication of '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus' in citrus plant. *Pytopathology* 99: 301–306.

Lopes SA, Frare GF (2008) Graft transmission efficiencies and multiplication of '*Candidatus* Liberibacter americanus'. *Plant Disease* 92: 21–24.

Lopes SA, Frare GF, Bertolini E, Cambra M, Fernandes NG, Ayres AJ, Marin DR, Bové JM (2009a) Liberibacters associated citrus Huanglongbing in Brazil: '*Candidatus* Liberibacter asiaticus' is heat tolerant, '*Candidatus* Liberibacter americanus' is heat sensitive. *Plant Disease* 93: 257–262.

Lopes SA, Frare GF, Bertolini E, Wulff NA (2008) Liberibacter populations in citrus and orange jasmine trees in the State Sao Paulo, Brazil. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 182–183, Orlando, Florida 2008. www.plantmanagementnetwork.org

Lopes SA, Frare GF, Yamamoto PT, Ayers AJ, Barbosa JC (2007) Ineffectiveness of pruning to control Huanglongbing caused by '*Candidatus* Liberibacter americanus'. *European Journal of Plant Pathology* 119: 463–468.

Lynch JM, Hobbie JE (1988) Micro-organisms in action: Concepts and Applications in Microbial Ecology. Blackwell Scientific Publications. pp. 109–110.

Manjunath KL, Halbert SE, Ramadugu C, Webb S, Lee RF (2008) Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus Huanglongbing in Florida. *Phytopathology* 98: 387–396.

Manjunath KL, Ramadugu C, Majil VM, Williams S, Irey M, Lee RF (2010) First report of the citrus Huanglongbing associated bacterium '*Candidatus* Liberibacter asiaticus from sweet orange, Mexican lime and Asian citrus psyllid in Belize. *Plant Disease* 94: 781.

Martinez Y, Llauger R, Batista L, Luis M, Iglesia A, Collazo C, Peńa I, Casin JC, Cueto J, Tablada LM (2008) First report of Candidatus 'Liberibacter asiaticus' associated with Huanglongbing in Cuba. *New Disease Reports* 18: 7. http://www.ndrs.org.uk/article.php?id=018007

Martinez AL, Nora DM, Price WC (1971) Observations on greening in the Philippines. *Animal Husbandry and Agricultural Journal* (March): 21–22.

Martinez AL, Wallace JM (1967) Citrus-leaf-Mottle-Yellows disease in the Philippines and Transmission of the causal virus by a psyllid, *Diaphorina citri*. *Plant Disease Reporter* 51: 692–695.

Martinez AL, Wallace JM (1968) Citrus leaf-mottle-yellows disease in the Philippines. *The Philippine Journal of Plant Industry* 33: 119–125.

Manjunath KL, Halbert SE, Ramadugu C, Webb S, Lee RF (2008) Detection of '*Candidatus* Liberibacter asiaticus' in *Diaphorina citri* and its importance in the management of citrus  Huanglongbing in Florida. *Phytopathology* 98: 387–396.

Mathur RN (1975) *Psyllidae of the Indian subcontinent*. New Delhi: Indian Council of Agricultural Research. 429 pp.

Matos L, Hilf ME, Camejo J (2009) First report of '*Candidatus* Liberibacter asiaticus' associated with citrus Huanglongbing in the Domanican Republic. *Plant Disease* 93: 668.

McClean APD (1970) Greening disease of sweet orange: its transmission in propagative parts and distribution in partially diseased trees. *Phytophylactica* 2: 263–268.

McClean APD (1974) The efficiency of citrus psylla, *Trioza erytreae* (Del G.) as a vector of greening disease of citrus. *Phytophylactica* 6: 45–54.

McClean APD, Oberholzer PCJ (1965a) Citrus psylla, a vector of the greening disease of sweet orange. *South African Journal of Agricultural Science* 8: 297–298.

McClean APD, Oberholzer PCJ (1965b) Greening disease of the sweet orange: evidence that it is caused by a transmissible virus. *South African Journal of Agricultural Science* 8: 253–276.

McClean APD, Schwarz RE (1970) Greening or blotchy-mottle disease of citrus. *Phytophylactica* 2: 177–194.

McFarland CD, Hoy MA (2001) Survival of *Diaphorina citri* (Homoptera: Psyllidae), and its two parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligharensis* (Hymenoptera: Encyrtidae), under different relative humidities and temperature regimes. *Florida Entomologist* 84: 227–233.

Mead FW (1976) The African citrus psyllid, *Trioza erytreae* (Del Guerico) (Homoptera: Psyllidae). *Entomology Circular No. 168*, 4 pp. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Mead FW (1977) The Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). *Entomology Circular No. 180*, 4 pp. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Mead FW (2008) Asian citrus psyllid – *Diaphorina citri* Kuwayama. Featured Creatures, University of Florida Institute of Food and Agricultural Sciences and Florida Department of Agriculture and Consumer Services. Online at http://creatures.ifas.ufl.edu/citrus/acpsyllid.htm (Accessed April 10).

Michaud JP (2004) Natural morality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biological Control* 29: 260–269.

Miyakawa T, Tsuno (1989) Occurrence of citrus greening disease in the Southern islands of Japan. *Annals Phytopathological Society of Japan* 55: 667–670.

Moll JN, Martin MM (1974) Comparison of the organism causing greening disease with several plant pathogenic Gram-negative bacteria, rickettsia-like organisms and mycoplasma-like organisms. *INSERM* 33: 89–96.

Moran VC (1968) The development of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae), on *Citrus limon* and four indigenous host plants. *Journal of the Entomological Society of Southern Africa* 31: 391–402.

Moran VC and Buchan PR (1975) Oviposition by the citrus psylla, *Trioza erytreae* (Homoptera: Psyllidae), in relation to leaf hardness *Entomologia Experimentalis et Applicata* 18: 96–104.

Moran VC, Blowers JR (1967) On the biology of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: PsyUidae). *J. Entomol. Soc. South. Afr.* 30: 96–106.

Mumford PM, Panggabean G (1982) A comparison of the effects of dry storage on seeds of citrus species. *Seed Science and Technology* 10: 256–266.

Nakata T (2006) Temperature-dependent development of the citrus psyllid, *Diaphorina citri* (Homoptera: Psylloidea), and the predicted limit of its spread based on overwintering in the nymphal stage in temperate regions of Japan. *Applied Entomology and Zoology* 41: 383–387.

NAPPO (2009a) Detección de Huanglongbing (*Candidatus* Liberibacter asiaticus) en el municipio de Tizimín, Yucatán, México. North American Plant Protection Organisation. http://www.pestalert.org/oprDetail.cfm?oprID=384 (Accessed 14 July 2009).

NAPPO (2009b) Expansion of Federal quarantine area for citrus greening due to the detection in the Commonwealth of Puerto Rico. North American Plant Protection Organisation. http://www.pestalert.org/oprDetail.cfm?oprID=407 (Accessed 1 December 2009).

NAPPO (2009c) First detection of Asian citrus psyllid *Diaphorina citri* in Arizona and new detection sites in California. North American Plant Protection Organisation. http://www.pestalert.org/oprDetail.cfm?oprID=411 (Accessed 5 January 2009).

NAPPO (2010) Confirmation of citrus greening (*Candidatus* Liberibacter asiaticus) in the Virgin Islands. North American Plant Protection Organisation. http://www.pestalert.org/oprDetail.cfm?oprID=430&keyword=candidatus (Accessed 13 July 2010).

Nariani TK, Ghosh SK, Kumar D, Raychaudhuri SP, Viswanath SM (1975) Detection and possibilities of therapeutic control of the greening disease of citrus caused by mycoplasmas. *Proceedings of Indian Nattional Science Acadamy Series B* 41: 334–39.

Nava DE, Torres MLG, Ridrigues MDL, Bento JMS, Parra JRP (2007) Biology of *Diaphorina citri* (Hem. Psyllidae) on different hosts and at different temperatures. *Journal of Applied Entomology* 131: 709–715.

Noble R, Roberts SJ (2004) Eradication of plant pathogens and nematodes during composting: a review. *Plant Pathology* 53: 548–568.

Oberholzer PCJ, von Standen DFA, Basson WJ (1965) Greening disease of sweet orange in South Africa. In: *Proceedings of the Third Conference of the International Organization of Citrus Virologists* (ed Price WC) pp. 213–219, Campinas, São Paulo, Brazil, September 1963. Gainesville: University of Florida Press.

Osman MS, Lim WH (1990) *Psylla murrayii* Math. (Fam. Psyllidae) on *Murraya koenigii* Spreng. (Fam. Rutaceae), a new pest record for W. Malaysia. In: *Proceedings of the Fourth International Asia Pacific Conference on Citrus Rehabilitation* (eds Aubert B, Tontyaporn S, Buangsuwon D) pp. 212–213, Chiang Mai, Thailand, February 1990. Rome: FAO UNDP.

Pande YD (1971) Biology of citrus psylla, *Diaphorina citri* Kuw. (Hemiptera: Psyllidae). *Israel Journal of Entomology* 6: 307–311.

Patt JM, Sétamou M (2010) Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its Rutaceous host plants. *Chemical Ecology* 39: 618–624.

Peel MC, Finlayson BL, McMahon T (2007) Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions* 4: 439–473.

Pietersen G, Arrebola E, Breytenbach JHJ, Korsten L, le Roux HF, la Grange H, Lopes SA, Meyer JB, Pretorius MC, Schwerdtfeger M, van Vuuren SP, Yamamoto P (2010) A survey for '*Candidatus* Liberibacter' species in South Africa confirms the poresence of only '*Ca*. L. africanus' in commercial citrus. *Plant Disease* 94: 244–249.

Pluke RWH, Qureshi JA, Stansly PA (2008) Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiate* (Hymenoptera: Eulophidae) in Puerto Rico. *Florida Entomologist* 91: 36–42.

Poe SR, Shea K (2007) Citrus greening and Asian citrus psyllid; availability of an environmental assessment. *United States Department of Agriculture Federal Register* 72: 62204–62205.

Polek M, Vidalakis G, Godfrey K (2007) Citrus Bacterial Canker Disease and Huanglongbing (Citrus Greening). Publication 8218. University of California, Division of Agriculture and Natural Resources, Exotic and Invasive Pests and Diseases Research Program. http://ccpp.ucr.edu/news/CitrusCanker&HuanglongbingDiseaseJan07.pdf. (Accessed 8 February 2008).

Postnikova EN, Stone AL, Wilson CM, Sherman DJ, Sechler A, Schuenzel EL, Schneider W, Damsteegt VD (2009) Preliminary evidence for mixed populations of '*Ca*. Liberibacter species' in Huanglongbing infections. *Phytopathology* 99 (Supplement): S205.

Pretorius MC, Van Vuuren SP (2006) Managing Huanglongbing (citrus greening disease) in the Western Cape. *South African Fruit Journal* 5: 59–62.

Qureshi JA, Stansly PA (2010) Dormant season foliar sprays of broad-spectrum insecticides: An effective component of integrated management for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus orchards. *Crop Protection* 29: 860–866.

Rae DJ, Liang WG, Watson DM, Beattie GAC, Huang MD (1997) Evaluation of petroleum spray oils for control of the Asian citrus psylla, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), in China. *International Journal of Pest Management* 43: 71–75.

Ramadugu C, Lopes S, Manjunath KL, Halbert S, Roose M, Lee RF (2008) Role of *Murraya* species in the spread of huanglongbing. In: In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 210, Orlando, Florida 2008. www.plantmanagementnetwork.org

Robles GP, Sanchez AH, Mendez LO (2008) National plan for detection of HLB in Mexico. In: *International Research Conference on Huanglongbing, Proceedings of the Meeting* p. 104, Orlando, Florida December 2008.

Rogers ME, Brlansky RH, Ebert TA, Serikawa RH, Schumann RA, Stelinski KP (2008) Acquisition of '*Candidatus* Liberibacter asiaticus' by the Asian citrus psyllid, *Diaphorina citri*, and the potential use of insecticides to prevent pathogen transmission. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 247–248, Orlando, Florida 2008. www.plantmanagementnetwork.org

Roistacher CN (1996) The economics of living with citrus disease: huanglongbing (greening) in Thailand. In: da Graça JV, Moreno P, Yokomi RK (eds), *Proceedings of the Thirteenth Conference of the International Organization of Citrus Virologists* (eds da Graça JV, Moreno P, Yokomi RK) pp. 279–285, Fuzhou, Fujian, China, November 1995. Riverside: International Organization of Citrus Virologists, University of California: Riverside.

Sadoyama Y, Takushi T (2008) The seasonal incidence of *Candidatus* Liberibacter asiaticus infection in the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in Okinawa, Japan. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 253–254, Orlando, Florida 2008. www.plantmanagementnetwork.org

Sakamaki Y (2005) Possible migration of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) between and within islands. *Occasional Papers of the Kagoshima University Research Centre* 42: 121–125.

Samways MJ, Manicom BQ (1983) Immigration, frequency distributions and dispersion patterns of the psyllid *Trioza erytreae* (Del Guárico) in a citrus orchard. *Journal of Applied Ecology* 20: 463–472.

Saponari M, Bac G. De, Breithaupt J, Loconsole G, Yokomi, R. K Catalano L (2010) First Report of 'Candidatus Liberibacter asiaticus' Associated with Huanglongbing in Sweet Orange in Ethiopia. *Plant Disease* 94: 482.

Schwarz RE, Green GC (1972) Heat requirements for symptom suppression and inactivation of the greening pathogen. In: Proceedings of the Fourth Conference of the International Organization of Citrus Virologists (Childs JFL ed.) pp. 44–51, FAO, Rome, Italy, October 1966. Gainesville: University of Florida Press.

Schwarz RE, Green GC (1970) Citrus greening and the citrus psyllid *Trioza erytreae*, a temperature-dependent agent-vector complex. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 79: 490–493.

Sechler A, Schuenzel EL, Cooke P, Donnua S, Thaveechai N, Postnikova E, Stone AL, Schneider WL, Damsteegt VD, Schaad NW (2009) Cultivation of '*Candidatus* Liberibacter asiaticus', '*Ca.* L. africanus', and '*Ca.* L. americanus' associated with Huanglongbing. *Phytopathology* 99: 480–486.

Shatters Jr RG (2008) Detection of '*Candidatus* Liberibacter asiaticus' in citrus seedlings germinated from Florida seed. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 198, Orlando, Florida 2008. www.plantmanagementnetwork.org

Shivankar VJ, Rao CN, Singh S (2000) Studies on citrus psylla, *Diaphorina citri* Kuwayama: a review. *Agricultural Review* 21: 199–204.

Song RL, Wu RJ, Ke C (1999) Elimination of the main citrus virus and virus-like diseases by shoot tip grafting. *Acta Phytopathologica Sinica* 29: 275–279.

Spann TM, Atwood RA, Yates JD, Rogers ME, Brlansky RH (2008) Dooryard Citrus Production: Citrus Greening Disease. IFAS Extension, University of Florida.

Stover E, Bowman K, McCollum G, Niedz R (2008) Developing transgenic solutions for HLB resistant citrus at the US Horticulture Research Laboratory. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 374, Orlando, Florida 2008. www.plantmanagementnetwork.org

Su HJ (2008) Research and Health Management of Citrus Huanglongbing in Taiwan. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 57–92, Orlando, Florida 2008. www.plantmanagementnetwork.org

Subandiyah S (1994) Transmission of citrus vein phloem degeneration (CVPD) to periwinkle through dodder. *Agrivita* 17: 21–22.

Sullivan M, Zink R (2007) Potential differences for HLB between Florida and the Western States. United States Department of Agriculture, Animal and Plant Health Inspection Service. Online at: http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/hlb.pdf (Accessed 20 February 2009).

Tatineni S, Sageram US, Gowda S, Robertson CJ, Dawson WO, Iwanami, Wang N (2008) In Planta Distribution of '*Candidatus* liberbacter asiaticus' as revealed by Polymerase Chain Reaction (PCR) and Real-time PCR. *Phytopathology* 98: 592–599.

Teixeira DC, Saillard C, Couture C, Martins EC, Wulff NA, Eveillard-Jagoueix S, Yamamoto PT, Ayres AJ, Bové JM (2008) Distribution and quantification of *Candidatus* Liberibacter americanus, agent of huanglongbing disease of citrus in São Paulo State, Brazil, in leaves of an affected sweet orange tree as determined by PCR. *Molecular and Cellular Probes* 22: 139–150.

Teixeira DDC, Dane JL, Eveillard S, Martins EC, de Jesus Junior WC, Yamamoto PT, Lopes SA, Bassanezi RB, Ayres AJ, Saillard C, Bové JM (2005b) Citrus huanglongbing in Sao Paulo State, Brazil: PCR detection of the '*Candidatus* Liberibacter species' associated with the disease. *Molecular and Cellular Probes* 19: 173–179.

Teixeira DDC, Saillard C, Eveillard S, Danet JL, Ayres AJ, da Costa PI, Bové JM (2005a) '*Candidatus* Liberibacter americanus', associated with citrus huanglongbing (greening disease) in Sao Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology* 55: 1857–1862.

Temu RPC, Andrew SM (2008) Endemism of plants in the Uluguru Mountains, Morogoro, Tanzania. *Forest Ecology and Management* 255: 2858–2869.

The Monitor (2009) Six-ton coriander shipment denied entry at Pharr bridge. November 27, 2009. http://www.themonitor.com/articles/pharr-32961-shipment-bridge.html (Accessed 30 November 2009).

The Packer (2009) Dogs detect more Asian citrus psyllids in California. http://thepacker.com/Dog-detects-more-Asian-citrus-psyllids-in-California/Article.aspx?oid=831685&aid=657&fid=PACKER-CROPS-AND-MARKETS Accessed September 2009.

Tirtawidjaja S (1981) Insect, dodder and seed transmissions of citrus vein phloem degeneration (CVPD). In: *4$^{Th}$ International Citrus Congress, Tokyo. International Society of Citriculture*, pp. 469–471.

Tomimura K, Miyata SI, Furuya N, Kubota K, Okuda M, Subandiyah S, Hung TH, Su HJ, Iwanami T (2009) Evaluation of genetic diversity among '*Candadatus* Liberibacter asiaticus' isolates collected in Southeast Asia. *Phytopathology* 99: 1062–1069.

Trivedi P, Sagaram US, Kim JS, Brlansky RH, Rogers ME, Stelinski LL, Oswalt C, Wang N (2009) Quantification of viable *Candidatus* Liberibacter asiaticus in hosts using quantitative PCR with the aid of ethidium monoazide (EMA). *European Journal of Plant Pathology* DOI 10.007/s10658-009-9439-x.

Tsagkarakis AE, Rogers ME (2008) Effects of host plant on fitness of the Asian citrus psyllid, *Diaphorina citri*. In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 242–243, Orlando, Florida 2008. www.plantmanagementnetwork.org

Tsai CH, Hung TH, Su HJ (2008) Strain identification and distribution of citrus Huanglongbing bacteria in Taiwan. *Botanical Studies* 49: 49–56.

Tsai JH (2006) Citrus greening and its psyllid vector. University of Minnesota, IPM World Textbook. Online at: http://ipmworld.umn.edu/chapters/TsaiGreening.htm (Accessed 8 March 2009).

Tsai JH, Liu YH (2000) Biology of *Diaphornia citri* (Homoptera: Psyllidae) on four host plants. *Horticultural Entomology* 93: 1721–1725.

Tsai CH, Su HJ, Liao YC Hung TH (2006) First Report of the Causal Agent of Huanglongbing ('*Candidatus* Liberibacter asiaticus') Infecting Kumquat in Taiwan. *Plant Disease* 90: 1360.

Tsai JH, Wang JinJun, Liu YingHong (2002) Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in Southern Florida. *Florida Entomologist* 85: 446–451.

UNCTAD (2006) Infocomm –Information on Citrus fruit. United Nations Conference on Trade and Development. Online at: http://www.unctad.org/infocomm/anglais/orange/market.htm (Accessed 8 February 2009).

Urasaki N, Kawano S, Mukai H, Uemori T, Takeda O, Sano T (2008) Detection of '*Candidatus* Liberibacter asiaticus' by cycleave isothermal and chimeric primer-initiated amplification of nucleic acids (Cycleave ICAN). In: *International Research Conference on Huanglongbing; Proceedings of the Meeting* (eds. Gottwald RT, Graham HJ) pp. 124–126, Orlando, Florida 2008. www.plantmanagementnetwork.org

USDA (2009) National Quarantine, Citrus Greening and Asian Citrus Psyllid. http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/nationalquarantinemap.pdf (Accessed 6 August 2009).

Van den Berg MA (1990) The citrus psylla, *Trioza erytreae* (Del Guárico) (Hemiptera: Triozidae): a review. *Agriculture, Ecosystems and the Environment* 30: 171–194.

Van den Berg MA, Anderson SH, Deacon VE (1991b) Population studies of the citrus psylla, *Trioza erytreae*: factors influencing dispersal. *Phytoparasitica* 19: 283–289.

Van den Berg MA, Deacon VE, Steenekamp PJ (1991a) Dispersal within and between citrus orchards and native hosts, and nymphal mortality of citrus psylla, *Trioza erytreae*. *Agriculture, Ecosystems and Environment* 35: 297–309.

Van den Berg MA, Van Vuuren VA, Deacon VE (1991–1992) Studies on greening disease transmission by the citrus psylla *Trioza erytreae* (Hemiptera: Triozidae). *Israel Journal of Entomology* 15–16: 51–56.

Van den Berg MA; Deacon VE (1988) Dispersal of the citrus psylla, *Trioza erytreae*, in the absence of its host plants. *Phytophylactica* 20: 361–368.

Van der Merwe CP (1941) The citrus psylla (*Spanioza erytreae*, del G.). *Science Bulletin 233, Entomology Series 8*, Department of Agriculture and Forestry, Union of South Africa. 12 pp.

Van Vuuren SP (1993) Variable transmission of African greening to sweet orange. In: *Proceedings of the Twelfth Conference of the International Organization of Citrus Virologists* (Moreno P, da Graça JV, Timmer LW) pp. 264–268, New Delhi, India, November 1992. Riverside: International Organization of Citrus Virologists, University of California, Riverside.

Van Vuuren SP, Moll JN, Wagner MJ (1986) The dynamics of greening transmission. In: *Proceedings of the Citrus and Subtropical Fruit Research Institute Symposium*, p. 48, October 1986, CSFRI, Nelspruit, South Africa.

Van Vuuren SP, Van der Merwe MJ (1992) The efficacy of citrus psylla, *Trioza erytreae*, as a vector of citrus greening disease. *Phytophylactica* 24: 258–288.

Wang LY, Hung SC, Hung TH, Su HJ (1996) Population fluctuation of *Diaphorina citri* Kuwayama and incidence of citrus likubin in citrus orchards in Chiayi area. *Plant Protection Bulletin* (Taipei) 38: 355–365.

Waterhouse DF (1998) *Biological Control of Insect Pests: Southeast Asian Pros*pects. ACIAR Monograph Series No. 51. Canberra: Australian Centre for International Agricultural Research.

Weinert MP, Jacobson SC, Grimshaw GJ, Bellis FA, Stephens PM, Gunua TG, Kame MF, Davis RI (2004) Detection of Huanglongbing (citrus greening disease) in Timor-Leste (East Timor) and Papua new Guinea. *Australasian Plant Pathology* 33: 135–136.

Wenninger EJ, Hall DG (2008) Importance of multiple mating to female reproductive output in *Diaphorina citri. Physiological Entomology* 33: 316–321.

Wenninger EJ, Stelinski LL, Hall DG (2008) Behavioral evidence for a female-produced sex attractant in *Diaphorina citri*. *Entomologia Experimentalis et Applicata* 128: 450–459, 2008

Wilkinson W (2007) *Pest exclusion advisory 21-2007*. Sacramento: State of California Department of Food and Agriculture.

Willits and Newcomb Inc (2004) Citrus seed General information http://www.wncitrus.com/pdfs/Rootstock%20Seed-Gen%20Info.pdf. (Accessed 9 July 2009).

WTO (1995) *Agreement on the application of sanitary and phytosanitary measures*. World Trade Organization, Switzerland.

Xie PH, Su CA, Lin ZG (1988) A study on the cold endurance of the Asian citrus psyllid in Zhejiang. In: *Proceedings of the Second Asian/Pacific Regional Workshop on citrus greening* (eds Aubert B, Ke C, Gonzales C) pp. 18–22, Lipa, Philippines, November 1988. Rome: UNDP-FAO.

Xu CF, Wang DX, Ke C (1991) Report of implementation of integrated control programme of citrus huanglungbing aiming at renovating old infected orchard in epidemic zone and protecting new non-infected orchard in non-epidemic zone. In: *Proceedings of the 6th International Asia Pacific Workshop on Integrated Citrus Health Management* (eds Ke C, Osman SB) pp. 55–61, Kuala Lumpur, Malaysia.

Xu CF, Xia YH, Li KB, Ke C (1988a) Further study of the transmission of citrus huanglongbing by a psyllid, *Diaphorina citri* Kuwayama. In: *Proceedings of the Tenth Conference of the International Organization of Citrus Virologists* (eds Timmer LW, Garnsey SM, Navarro L) pp. 243–248. Valencia, Spain, November 1986. Riverside: International Organization of Citrus Virologists, University of California: Riverside.

Xu CF, Xia YH, Li KB, Ke C (1988b) Studies on the law of transmission of citrus huanglungbin by psyllid, *Diaphorina citri* and the distribution of the pathogen. *Journal of the Fujian Academy of Agricultural Science* 3: 57–62.

Yang CK, Li FS (1984) Nine new species and a new genus of psyllids from Yunnan. *Entomotaxonomia* 6: 251–266.

Yang Y, Huang M, Andrew G, Beattie C, Xia Y, Ouyang G, Xiong J (2006) Distribution, biology, ecology and control of the psyllid *Diaphorina citri* Kuwayama, a major pest of citrus: A status report for China. *International Journal of Pest Management* 52: 343–352.

Yuma Sun (2009) Yuma citrus production area to be under quarantine.

Zhang M.Q, Duan YP, Zhou LJ, Turechek WW, Stover E, Powell CA(2010) Screening molecules for control of citrus huanglongbing using an optimized regeneration system for '*Candidatus* Liberibacter asiaticus'-infected periwinkle (*Catharanthus roseus*) cuttings. *Phytopathology* 100: 239–245.

Zhao XY (1981) Citrus yellow shoot disease (huanglongbing) in China – a review. In: *Proceedings of the Fourth International Society Citriculture Congress* (ed Matsumoto K) pp. 466–469. Tokyo, Japan, November 1981. Riverside: International Society of Citriculture.

Zhou BB, Yin YP, Hu H, Wang ZK (2008a) Genetic diversity of '*Candidatus* Liberibacter asiaticus' isolates from different geographical regions in China. In: *Abstracts and Proceedings of the Eleventh International Citrus Congress*, p. 55, Wuhan, China, October 2008.

Zhou L, Duan Y, Gabriel D, Gottwald TR (2008b) Seed transmission of *Candidatus* Liberibacter asiaticus in periwinkle and dodder resulted in low bacterial titre and very mild disease in periwinkle. *Phytopathology* 98: S181.

Zhou LJ, Gabriel DW, Duan YP, Halbert SE, Dixon WN (2007) First report of dodder transmission of huanglongbing from naturally infected *Murraya paniculata* to Citrus. *Plant Disease* 91: 227.