

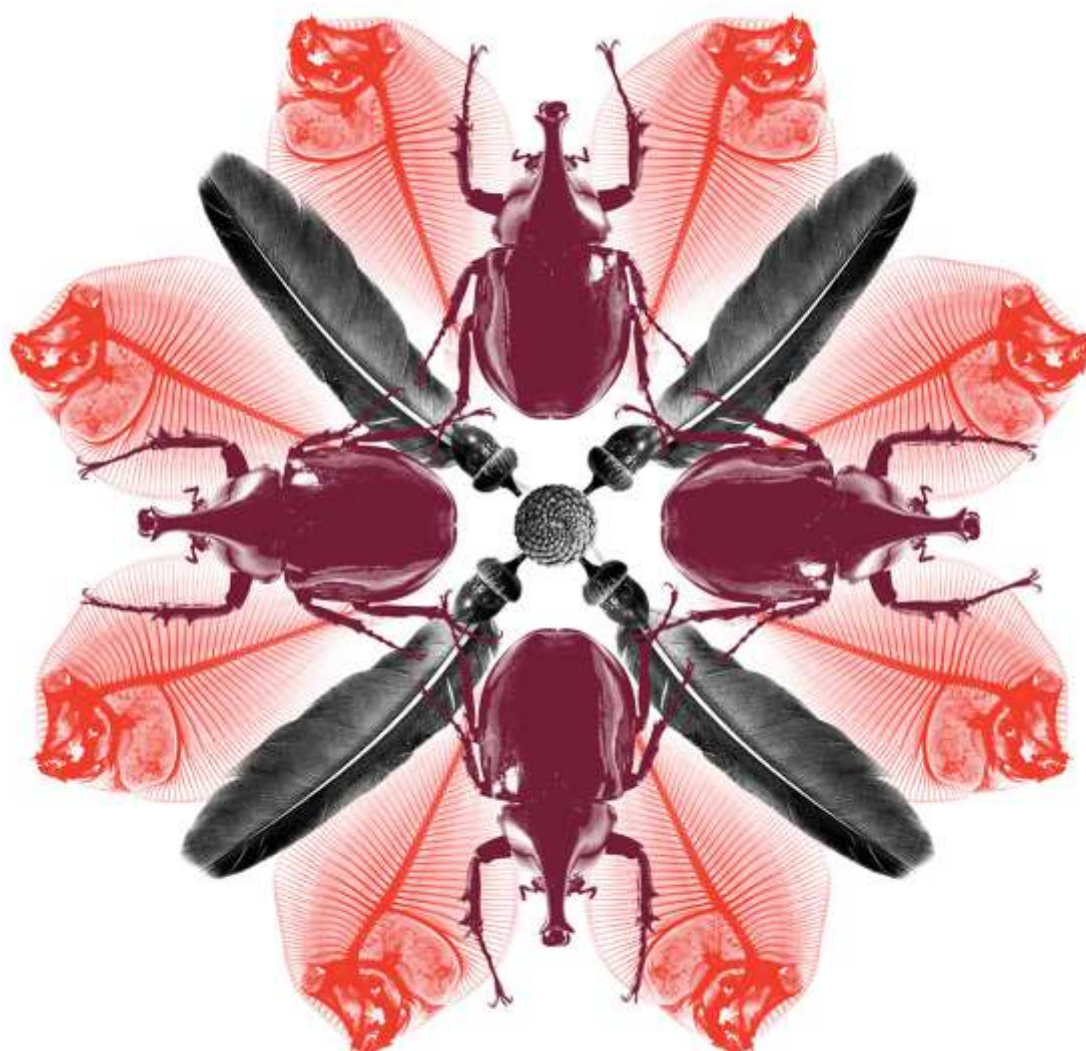


Australian Government  
Department of Agriculture  
and Water Resources

# Draft pest risk analysis for '*Candidatus Liberibacter solanacearum*' associated with apiaceous crops

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December 2015



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Australian Government Department of Agriculture and Water Resources  
GPO Box 858 Canberra ACT 2601

Switchboard: +61 2 6272 3933 or 1800 900 090

Facsimile: +61 2 6272 3307

Email: [plant@agriculture.gov.au](mailto:plant@agriculture.gov.au)

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### **Stakeholder submissions on draft reports**

This draft report has been issued to give all interested parties an opportunity to comment on relevant technical biosecurity issues, with supporting rationale. A final report will then be produced taking into consideration any comments received.

Submissions should be sent to the Australian Government Department of Agriculture and Water Resources following the conditions specified within the related Biosecurity Advice, which is available at: [agriculture.gov.au/ba/memos](http://agriculture.gov.au/ba/memos)

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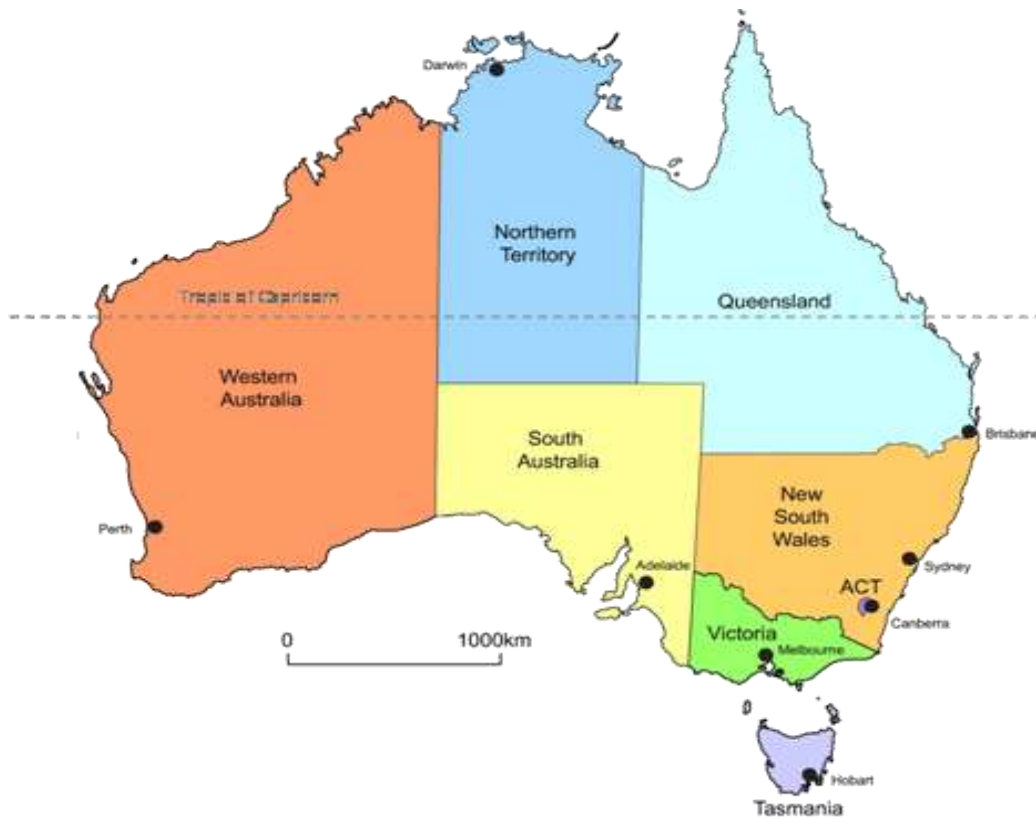
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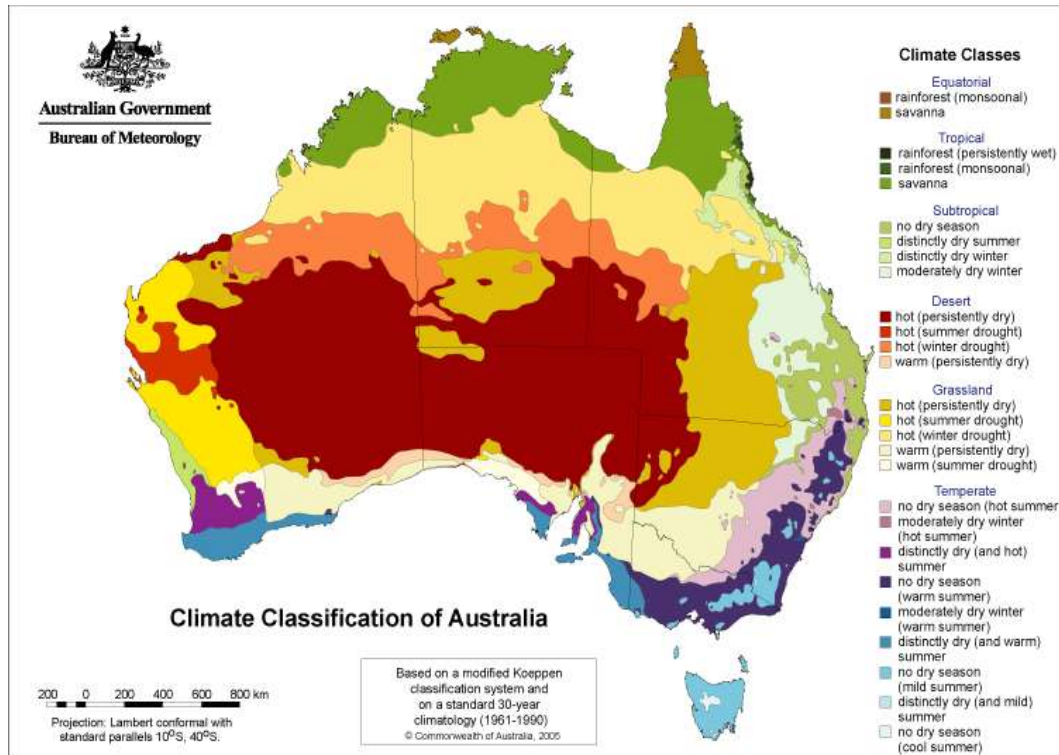
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Map 1 Map of Australia



Map 2 A guide to Australia's bio-climatic zones





## Acronyms and abbreviations

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<b>Term or abbreviation</b>	<b>Definition</b>
ACT	Australian Capital Territory
ALOP	Appropriate level of protection
FAO	Food and Agriculture Organization of the United Nations
ICON	The Australian Government Department of Agriculture and Water Resources Import CONditions database
IPC	International Phytosanitary Certificate
IPPC	International Plant Protection Convention
IRA	Import risk analysis
ISPM	International Standard for Phytosanitary Measures
' <i>Ca. L. solanacearum</i> '	' <i>Candidatus Liberibacter solanacearum</i> '
NSW	New South Wales
NPPO	National Plant Protection Organisation
NT	Northern Territory
PEQ	Post-entry quarantine
PCR	Polymerase Chain Reaction
PRA	Pest risk analysis
Qld	Queensland
SA	South Australia
SEM	Scanning Electron Microscopy
SPS	Sanitary and Phytosanitary
TAS	Tasmania
TEM	Transmission Electron Microscopy
VIC	Victoria
WA	Western Australia
WTO	World Trade Organization

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## Summary

The Australian Government Department of Agriculture and Water Resources (the department) initiated this pest risk analysis (PRA) in response to the introduction of emergency measures to manage '*Candidatus Liberibacter solanacearum*' ('*Ca. L. solanacearum*') infecting apiaceous crops, including carrot (*Daucus carota*) and celery (*Apium graveolens*). This bacterium is not known to occur in Australia and is reported to cause serious damage to the carrot and celery industries in Europe. Australia introduced emergency measures on apiaceous host propagative material on 20 October 2014 to manage the introduction of '*Ca. L. solanacearum*' into Australia.

The International Plant Protection Convention (IPPC) and the 'World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures' (SPS Agreement) requires that any phytosanitary measures against the introduction of new pests must be technically justified. The IPPC's International Standards for Phytosanitary Measures (ISPM) 1 states that 'countries may take appropriate emergency action on a pest posing a potential threat to its territories; however, it requires that the action be evaluated as soon as possible to justify the continuance of the action'. Therefore, this PRA meets Australia's international obligations to review the emergency phytosanitary measures on '*Ca. L. solanacearum*' associated with apiaceous crops.

This PRA justifies Australia's decision to introduce emergency measures against '*Ca. L. solanacearum*' for apiaceous host propagative material. The department considers that the current emergency measures are adequate to mitigate the risk posed by '*Ca. L. solanacearum*' associated with carrot and celery propagative material. Therefore, these emergency measures are proposed to become the standard conditions to import carrot and celery propagative material into Australia. More recently, parsnip was also reported as natural host of '*Ca. L. solanacearum*'. Consequently, these measures are extended to parsnip tissue cultures. Proposed changes to the emergency measures include the option for small seed lots to be tested off-shore; and alternative conditions for tissue cultures of unknown health status.

The ultimate goal of Australia's phytosanitary measures is to protect plant health and manage the introduction of '*Ca. L. solanacearum*' associated with apiaceous crops. The proposed import conditions for carrot, celery and parsnip propagative material are summarized below.

**Seeds for sowing (carrot):** mandatory off-shore or on-shore molecular testing **OR** mandatory off-shore or on-shore heat treatment; **AND** a Phytosanitary Certificate with the additional declaration that the mandatory treatment or testing has been conducted in accordance with Australia's requirements.

**Tissue cultures (carrot, celery and parsnip) of known health:** mandatory off-shore molecular testing; **AND** a Phytosanitary Certificate with the additional declaration that the testing has been conducted in accordance with Australia's requirements.

**Tissue cultures (carrot, celery and parsnip) of unknown health:** mandatory growth in a closed government post-entry quarantine (PEQ) facility for disease screening; **AND** mandatory on-shore PCR testing for freedom from '*Ca. L. solanacearum*'.

The department invites comments on the technical aspects of the proposed risk management measures within the consultation period. In particular, comments are sought on their appropriateness and any other measures stakeholders consider would provide equivalent risk management outcomes. The department will consider any comments received before finalising the PRA and import 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 entering, establishing and spreading in Australia, thereby threatening Australia's agricultural industries and the environment.

The risk analysis 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 importing 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. However, 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 stringent, 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 pest risk analyses (PRAs) are undertaken by the Department of Agriculture and Water Resources, hereafter referred to as the department, using teams of technical and scientific experts in relevant fields and involves consultation with stakeholders at various stages during the process.

Further information about Australia's biosecurity framework is provided in the *Import Risk Analysis Handbook 2011* located on the [Department of Agriculture](#) and Water Resources website.

### 1.2 This pest risk analysis

The International Plant Protection Convention (IPPC) and the 'World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures' (SPS Agreement) requires that any phytosanitary measures against the introduction of new pests must be technically justified. Therefore, the department undertook this pest risk analysis (PRA) to meet Australia's obligations under the IPPC and the International Standards for Phytosanitary Measures (ISPM) 1 to review emergency phytosanitary measures introduced to manage the risk of introducing '*Candidatus Liberibacter solanacearum*' ('*Ca. L. solanacearum*') haplotypes associated with apiaceous crops into Australia through legal pathways. Australia introduced emergency measures for seed (carrot) and tissue cultures (carrot, celery) imports and notified trading partners of the emergency measures in August 2014 through a World Trade Organization Sanitary and Phytosanitary (WTO SPS) notification (G/SPS/N/AUS/345).

#### 1.2.1 Background

The association of '*Ca. L. solanacearum*' with carrot crops was reported in 2010 in Finland (Munyanzeza et al. 2010a) and since then the bacterium has spread to several carrot growing areas in Europe (Haapalian 2014) and North Africa (Tahzima et al. 2014); and celery growing regions of Spain (Teresani et al. 2014a) and Austria (EPPO Reporting Service 2015). The

confirmation of the seed-borne nature and seed transmission of '*Ca. L. solanacearum*' in carrot (Bertolini et al. 2014) was a new phytosanitary situation. Therefore, Australia introduced emergency measures for apiaceous host propagative material notified trading partners of the emergency measures on 21 August 2014 through a World Trade Organization Sanitary and Phytosanitary (WTO SPS) notification (G/SPS/N/AUS/345).

Prior to the introduction of emergency measures, importation of propagative material of apiaceous crops (tissue cultures, seed) from all sources was allowed entry into Australia without any specific disease testing.

### **'*Candidatus Liberibacter solanacearum*' associated with apiaceous crops**

2010: **Finland:** Carrot plants with symptoms resembling those of carrot psyllid (*Trioza apicalis*) damage were observed in commercial fields in southern Finland in 2008. Molecular tests indicated that '*Ca. L. solanacearum*' was present in the infected plants (Munyaneza et al. 2010a).

2012: **Norway:** '*Ca. L. solanacearum*' detected in carrot fields in four provinces (Munyaneza et al. 2012b). The carrot psyllid, *Trioza apicalis*, was found to be associated with '*Ca. L. solanacearum*' in Norway (Munyaneza et al. 2012b).

**Sweden:** '*Ca. L. solanacearum*' detected in carrot in Halland province (Munyaneza et al. 2012a). The carrot psyllid, *Trioza apicalis*, was found to be associated with '*Ca. L. solanacearum*' in Sweden (Munyaneza et al. 2012a).

**Spain:** '*Ca. L. solanacearum*' was confirmed from mainland Spain (Alicante, Albacete and Valencia) in carrot crops sampled between 2008 and 2010 (Alfaro-Fernández et al. 2012a). '*Ca. L. solanacearum*' was also detected in carrot crops sampled between 2009 and 2010 in the Canary Islands (Alfaro-Fernández et al. 2012b). '*Candidatus Liberibacter solanacearum*' in carrot crops was found to be associated with the psyllid, *Bactericera trigonica*, in the Canary Islands and mainland Spain (Alfaro-Fernández et al. 2012a, b; EPPO 2012).

2014: **Spain:** '*Ca. L. solanacearum*' was detected in celery in Villena (Alicante, Spain) (Teresani et al. 2014b).

The seed-borne nature and seed to seedling transmission of '*Ca. L. solanacearum*' was confirmed only in carrot seeds (Bertolini et al. 2014).

**France:** '*Ca. L. solanacearum*' was reported to be detected in carrot seed producing crops in central France (Loiseau et al. 2014).

**Morocco:** '*Ca. L. solanacearum*' was detected in carrot crops (Tahzima et al. 2014). This was the first report of '*Ca. L. solanacearum*' in Africa. The psyllid vector has not been identified in carrot crops in Morocco.

**Australia:** The department introduced emergency measures for imported seeds (carrot) and tissue cultures (carrot and celery) to mitigate the risk of introducing '*Ca. L. solanacearum*' into Australia on these pathways.

2015: **Austria:** '*Ca. L. solanacearum*' was detected in carrot and celery crops (EPPO Reporting Service 2015). Infected plants were destroyed and the bacterium in Austria is declared as transient, actionable and under surveillance.

**Germany:** '*Ca. L. solanacearum*' was detected in commercial carrot fields in Lower Saxony (Munyanenza et al. 2015). '*Candidatus Liberibacter solanacearum*' in carrot was found to be associated with the carrot psyllid, *Trioza apicalis*, in Germany (Munyanenza et al. 2015).

**Spain:** '*Ca. L. solanacearum*' was detected in commercial parsnip fields (Cambra et al. 2015).

Under ISPM 1: *Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade*, Australia is required to review emergency phytosanitary measures and conduct a science-based assessment, such as a PRA, to justify the continuance of the measures. Therefore, Australia initiated this pest risk analysis to justify emergency measures against '*Ca. L. solanacearum*' haplotypes associated with apiaceous (carrot, celery) propagative material.

### 1.2.2 Scope

The scope of this PRA includes:

- assessing the risk of introducing '*Ca. L. solanacearum*' associated with Apiaceae (carrot, celery and parsnip) propagative material (seeds and tissue cultures only) from all sources;
- reviewing and evaluating the existing risk management measures (including emergency measures) for the identified risks; and
- proposing additional risk management measures where appropriate.

This PRA does not consider existing phytosanitary measures during the pest risk assessment. Existing phytosanitary measures are only considered during the development of risk management measures, if they are required, following the pest risk assessment.

This PRA does not assess the risk of introducing '*Ca. L. solanacearum*' in infected psyllids. Appropriate measures, including fumigation with methyl bromide, are already in place to mitigate the risk of accidental introduction of these insect pests with their host material.

This PRA is limited to recommending appropriate phytosanitary measures to address the risk of introducing '*Ca. L. solanacearum*' associated with apiaceous propagative material into Australia. It is the importer's responsibility to ensure compliance with the requirements of all other regulatory and advisory bodies associated with importing commodities to Australia. These include the Australian Department of Immigration and Border Protection, Department of Health, Therapeutic Goods Administration, Australian Pesticides and Veterinary Medicines Authority, Department of the Environment and state and territory departments of agriculture.

### 1.2.3 Consultation

The department worked closely with industry stakeholders during the development of emergency measures to minimise any trade and crop production disruption. Prior to introducing emergency measures against '*Ca. L. solanacearum*', the department consulted with the seed industry including AusVeg, the Australian Seed Federation and representatives of domestic and international seed companies.

- 16 June 2014 The department held a teleconference with industry to discuss the implications of the published evidence that '*Candidatus Liberibacter solanacearum*' is seed-borne and seed-transmissible in carrot. The department stated that, based on the current information, future carrot seed imports would be regulated from all sources and that it intended to introduce the regulatory measures within four to six weeks.
- 10 July 2014 The department held a teleconference with industry to discuss the intention of implementing emergency measures for '*Candidatus Liberibacter solanacearum*' on imported carrot seeds. The department stated that, based on the current information, future carrot seed imports would be regulated from all sources and that it intended to introduce the regulatory measures within four to six weeks.
- 5 August 2014 The department held a teleconference with industry to discuss the proposed emergency measures for future imports of carrot (seed, tissue culture) and celery (tissue culture) from all sources.
- 21 August 2014 Australia notified trading partners of the upcoming emergency measures through a WTO SPS notification (G/SPS/N/AUS/345), advising that the measures would take effect from 20 October 2015.
- 22 August 2014 The department individually notified National Plant Protection Organisations of the main trading partners affected by the emergency measures, including Belgium, France, Germany, Italy, Japan, the Republic of Korea, Malaysia, the Netherlands, New Zealand, Singapore, the United Arab Emirates, the United Kingdom and the United States of America.
- 25 August 2014 The department published an ICON alert advising importers of the planned emergency measures for carrot (seed, tissue culture) and celery (tissue culture), to take effect from 20 October 2014.
- 20 October 2014 Australia's emergency measures came into force for imports of carrot (seed, tissue culture) and celery (tissue culture) from all sources.
- The department published an ICON alert advising importers of the commencement of the emergency measures for carrot (seed, tissue culture) and celery (tissue culture). The ICON alert included details on a transition period for carrot seeds in transit.
- The department advised industry representatives that the import conditions for carrot seed had been updated in ICON.
- 21 January 2015 The department published an ICON alert advising importers that carrot seeds require an Import Permit.
- 27 August 2015 The department provided an update on the progress of the '*Candidatus Liberibacter solanacearum*' draft PRA at the Australian Seed Federation Annual Conference held in Toowoomba, Australia.

## 2 Method for pest risk analysis

The Department of Agriculture and Water Resources 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* (FAO 2013) that have been developed under the SPS Agreement (WTO 1995).

Phytosanitary terms used in this PRA are defined in ISPM 5: *Glossary of phytosanitary terms* (FAO 2015). A glossary of the terms used is provided at the back of this report.

The PRA is conducted in the following three consecutive stages: initiation, pest risk assessment and pest risk management.

### 2.1 Stage 1: Initiation of the Pest Risk Analysis (PRA)

According to ISPM 2 (FAO 2007), a PRA process may be initiated as a result of:

- identification of a pathway that presents a potential pest risk (a means of pest introduction or spread);
- identification of a pest that may require phytosanitary measures (a pest may have been detected or intercepted, a request made to import it or it may have been reported elsewhere);
- review or revision of existing phytosanitary policies and priorities; or
- identification of an organism not previously known to be a pest.

Australia introduced emergency measures in October 2014 in response to reports of '*Ca. L. solanacearum*' being associated with apiaceous crops (carrot, celery) and being seed-borne in carrot (Bertolini et al. 2014). In accordance with ISPM 2 (FAO 2007) this PRA was initiated by the department as a basis for a review and possible revision of the emergency measures introduced by Australia for carrot (seed, tissue cultures) and celery (tissue cultures) for propagation into Australia. In Australia, '*Ca. L. solanacearum*' associated with apiaceous crops (carrot and celery) have been regulated as a quarantine pest since October 2014.

In the context of this PRA, the natural hosts of '*Ca. L. solanacearum*' (Table 1) are a potential import 'pathway' by which '*Ca. L. solanacearum*' may enter Australia.

**Table 1 Potential pathways by which '*Ca. L. solanacearum*' may enter Australia**

Scientific name	Reference	Seed-borne	Potential pathways
<i>Apium graveolens</i> (celery)	Teresani et al. 2014a; EPPO Reporting Service 2015		Tissue culture
<i>Daucus carota</i> (carrot)	Munyaneza et al. 2010a,b	Yes (Bertolini et al. 2014)	Tissue culture, Seed
<i>Pastinaca sativa</i> (parsnip)	Cambra et al. 2015		Tissue culture

For this PRA, the 'PRA area' is defined as Australia for '*Ca. L. solanacearum*' haplotypes associated with apiaceous crops (carrot, celery and parsnip) and their vectors.



## 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 magnitude of the associated potential economic consequences' (FAO 2015). The pest risk assessment provides technical justification for identifying quarantine pests and for establishing phytosanitary import requirements.

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

- pest categorisation;
- assessment of the probability of entry, establishment and spread; and
- assessment of potential consequences.

### 2.2.1 Pest categorisation

Pest categorisation identifies which of the pests with the potential to be on the commodity are quarantine pests for Australia and require a pest risk assessment. 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 (FAO 2015).

The process of a pest categorisation is summarised by ISPM 11 (FAO 2013) as a screening procedure based on the following criteria:

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

### 2.2.2 Assessment of the likelihood 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 2013). The SPS Agreement (WTO 1995) uses the term likelihood rather than probability for these estimates. In qualitative PRAs, the Australian Government Department of Agriculture and Water Resources uses the term 'likelihood' for the descriptors it uses for its estimates of likelihood of entry, establishment and spread. The use of the term 'probability' is limited to the direct quotation of ISPM definitions. A summary of this process is given below, followed by a description of the qualitative methodology used in this PRA.

#### Likelihood of entry

The likelihood of entry describes the likelihood 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. Assessing the likelihood of entry requires an analysis of each of the pathways with which a pest may be associated, from its origin to its distribution in the PRA area.

For the purpose of considering the likelihood of entry, the department divides this step into two components:

- **Likelihood of importation:** the likelihood that a pest will arrive in Australia when a given commodity is imported.
- **Likelihood of distribution:** the likelihood 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 host.

Factors considered in the likelihood of importation include the:

- 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;
- mode of trade (for example, bulk or packed);
- 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; and
- commercial procedures (for example, refrigeration) applied to consignments during transport and storage in the country of origin, and during transport to Australia.

Factors considered in the likelihood of distribution include the:

- commercial procedures (for example, 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 importation takes place;
- intended use of the commodity (for planting, processing or consumption); and
- risks from by-products and waste.

### **Likelihood of establishment**

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO 2015). In order to estimate the probability of establishment of a pest, reliable biological information (lifecycle, host range, epidemiology and survival) 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 likelihood of establishment in the PRA area include the:

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

### **Likelihood of spread**

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO 2015). The likelihood 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 likelihood 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 carefully compared with that in the areas where the pest currently occurs and expert judgement is used to assess the likelihood of spread.

Factors considered in the likelihood of spread include the:

- 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; and
- potential vectors of the pest in the PRA area.

### **Assigning likelihoods for entry, establishment and spread**

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). Descriptive definitions for these descriptors and their indicative ranges are given in Table 2. The indicative likelihood ranges are only provided to illustrate the boundaries of the descriptors and are not used beyond this purpose in qualitative PRAs. These indicative likelihood ranges provide guidance to the risk analyst and promotes consistency between different pest risk assessments.

**Table 2 Nomenclature of likelihoods**

<b>Likelihood</b>	<b>Descriptive definition</b>	<b>Indicative range</b>
High	The event would be very likely to occur	0.7 < to ≤ 1
Moderate	The event would occur with an even likelihood	0.3 < to ≤ 0.7
Low	The event would be unlikely to occur	0.05 < to ≤ 0.3
Very low	The event would be very unlikely to occur	0.001 < to ≤ 0.05
Extremely low	The event would be extremely unlikely to occur	0.000001 < to ≤ 0.001
Negligible	The event would almost certainly not occur	0 < to ≤ 0.000001

## Combining likelihoods

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 3). This matrix is then used to combine the likelihood of entry and the likelihood of establishment, and then the likelihood of entry and establishment is combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

For example, if the likelihood of importation is assigned a descriptor of 'low' and the likelihood of distribution is assigned a descriptor of 'moderate', then they are combined to give a likelihood of 'low' for entry. Then if the likelihood of establishment has been assigned a descriptor of 'high', this will be combined with the likelihood of entry (low), to give a likelihood for entry and establishment of 'low'. The assigned likelihood for spread (for example 'very low') would then be combined with the likelihood for entry and establishment (low), to give an overall likelihood for entry, establishment and spread of 'very low'. This can be summarised as:

importation x distribution = entry [E]

**low x moderate = low**

entry [E] x establishment = [EE]

**low x high = low**

[EE] x spread = [EES]

**low x very low = very low**

**Table 3 Matrix of rules for combining 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

### **Time and volume of trade**

One factor affecting the likelihood of entry is the volume and duration of trade. If all other conditions remain the same, the overall likelihood of entry will increase as time passes and the overall volume of trade increases.

The Department of Agriculture and Water Resources normally considers the likelihood of entry on the basis of the estimated volume of one year's trade. However, in case of a high risk commodity, the volume of trade is restricted to certain numbers. Therefore, other factors listed in ISPM 11 (FAO 2013) may not be relevant to propagative material of a high risk commodity.

### **2.2.3 Assessment of potential consequences**

The objective of the consequence assessment is to provide a structured and transparent analysis of the potential consequences if the pest 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 2015) and ISPM 11 (FAO 2013).

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

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

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

- eradication or control;
- domestic trade;
- international trade; and
- the 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, 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.

The estimates of the magnitude of the potential consequences over the four geographic levels were translated into a qualitative impact score (A–G) using Table 4. For example, a consequence with a magnitude of 'significant' at the 'district' level will have a consequence impact score of D.

**Table 4 Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales**

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

Note: In earlier qualitative PRAs, 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 to F has been 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 5 were adjusted accordingly.

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 5). These rules are mutually exclusive, and are assessed in numerical order until one applies.

**Table 5 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 more than one criterion has an impact of 'F'; or 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 all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B'; and all remaining criteria have an impact of 'A'.	Negligible

#### 2.2.4 Estimation of the unrestricted risk

Once the the assessment of the likelihood of entry, establishment and spread and for potential consequences 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 6) to combine the estimates of the likelihood of entry, establishment, spread and the overall consequences of pest establishment and spread. Therefore, risk is the product of likelihood and consequence.

**Table 6 Risk estimation matrix**

Likelihood of pest entry, establishment and spread	Consequences 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

When interpreting the risk estimation matrix, note the descriptors for each axis are similar (for example, 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.

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

The SPS Agreement (WTO 1995) defines the concept of an ‘appropriate level of sanitary or phytosanitary protection (ALOP)’ as the level of protection deemed appropriate by a World Trade Organisation (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 providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table 5 marked ‘very low risk’ represents Australia’s ALOP.

## 2.3 Stage 3: Pest risk management

Pest risk management evaluates and selects risk management options to reduce the risk of entry, establishment or spread of identified pests for the identified import pathways. To effectively prevent the introduction of pests associated with an identified pathway, a series of important safeguards, conditions or phytosanitary measures must be in place. Propagative material represents a direct pathway for pests identified by pest categorisation. This pathway is direct since the end-use is the planting of a known host plant.

### 2.3.1 Identification and selection of appropriate risk management options

Phytosanitary measures to prevent the establishment and spread of quarantine pests may include any combination of measures, including pre- or post-harvest treatments, inspection at various points between production and final distribution, surveillance, official control, documentation, or certification. A measure or combination of measures may be applied at any



one or more points along the continuum between the point of origin and the final destination. Pest risk management explores options that can be implemented (i) in the exporting country, (ii) at the point of entry or (iii) within the importing country. The ultimate goal is to protect plants and prevent the introduction of identified quarantine pests.

Examples of phytosanitary measures which may be applied to propagative material consignments include:

- **Import from pest free areas only (ISPM 4, 10)**—the establishment and use of a pest free area by a National Plant Protection Organisation (NPPO) provides for the export of plants from the exporting country to the importing country without the need for the application of additional phytosanitary measures when certain requirements are met.
- **Testing for freedom from regulated pests**—this is a practical measure for visible pests or for pests which produce visible symptoms on plants.
- **Inspection and certification (ISPM 7, 12, 23)**—the exporting country may be asked to inspect the shipment and certify that the shipment is free from regulated pests before export.
- **Specified conditions for preparation of the consignment**—the importing country may specify steps that must be followed in order to prepare the consignment for shipment. These conditions can include the requirement for plants to be produced from appropriately tested parent material.
- **Pre-entry or post-entry quarantine**—the importing country may define certain control conditions, inspection and possible treatment of shipments upon their entry into the country. Pre- or post-entry quarantine of dormant cuttings, seeds and even tissue cultures (*in vitro* plantlets) can help avoid the introduction of new viruses or allied pathogens into the importing countries.
- **Removal of the pest from the consignment by treatment or other methods**—the importing country may specify chemical or physical treatments that must be applied to the consignment before it may be imported.

Measures can range from total prohibition to permitting imports subject to visual inspection. In some cases, more than one phytosanitary measure may be required in order to reduce the pest risk to an acceptable level.

### 3 The pathogen

The genus '*Candidatus Liberibacter*' ('*Ca. L.*') [Rhizobiales: Rhizobiaceae] is composed of gram-negative bacteria belonging to the alpha subdivision of the proteobacteria (Jagoueix et al. 1996; Bové 2006). '*Candidatus Liberibacter*' species are fastidious, phloem-limited and infect a variety of agriculturally important crops (Haapalainen 2014). These 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). These bacteria are naturally transmitted between plants by psyllids, which feed on plant phloem sap (Janse 2012).

#### 3.1 '*Candidatus Liberibacter*' species

The genus '*Candidatus Liberibacter*' contains six species that differ in their vector specificity, environmental tolerances and host ranges (Bové 2006; Lopes & Frare 2008). Characteristics of these species are summarized in Table 7.

**Table 7 Characteristics of '*Candidatus Liberibacter*' species**

<b>'<i>Candidatus Liberibacter</i>' species</b>	<b>Natural host plant</b>	<b>Area of distribution</b>	<b>Temperature sensitivity</b>
' <i>Ca. L. africanus</i> '	Rutaceae (Bové 2006)	Sub-Saharan Africa, Saudi Arabia, Yemen	Heat sensitive (Bové et al. 1974)
' <i>Ca. L. americanus</i> '	Rutaceae (Bové 2006)	Brazil	Heat sensitive (Lopes et al. 2009)
' <i>Ca. L. asiaticus</i> '	Rutaceae (Bové 2006)	Southern Asia, Brazil, Caribbean, USA, Africa, Saudi Arabia, Yemen	Heat tolerant (Bové 2006)
' <i>Ca. L. crescens</i> '	Caricaceae (Leonard et al. 2012)	Puerto Rico (Caribbean)	Heat sensitive (Haapalainen 2014)
' <i>Ca. L. europaeus</i> '	Rosaceae (Camerota et al. 2012); Fabaceae (Thompson et al. 2013)	Europe Europe, New Zealand	Heat sensitive (Haapalainen 2014)
' <i>Ca. L. solanacearum</i> '	Solanaceae (Hansen et al. 2008; Munyaneza 2012); Apiaceae (Teresani et al. 2014b; Bertolini et al. 2014)	North and Central America, New Zealand Northern Europe, Austria, France, Germany, Morocco, Spain	Heat sensitive (Munyaneza 2012)

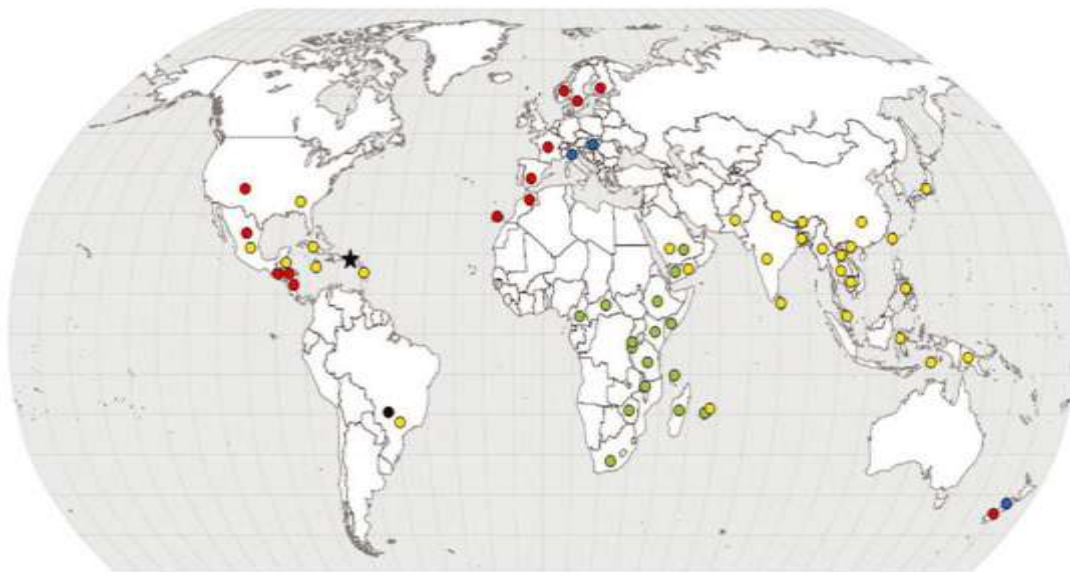
'*Candidatus Liberibacter*' species are associated with Huanglongbing, also referred to as Citrus greening (Bové 2006), Zebra chip disease (Hansen et al. 2008) and other diseases of crops from the families of Apiaceae (Munyaneza et al. 2010a; Teresani et al. 2014b), Caricaceae (Leonard et al. 2012), Fabaceae (Thompson et al. 2013), Rosaceae (Camerota et al. 2012), Rutaceae (Bové 2006) and Solanaceae (Liefting et al. 2009b; Wen et al. 2009; Ling et al. 2011).

'*Candidatus Liberibacter*' species affecting solanaceous crops were confirmed in New Zealand in 2008 (Liefting et al. 2009a) and later on, the pathogen associated with 'Zebra chip' disease in potatoes was identified as a '*Ca. L.*' species (Abad et al. 2009; Liefting et al. 2009a). Molecular studies confirmed that the American isolates of '*Ca. L.*' represented the same species found in New Zealand and the name '*Ca. L. solanacearum*' was suggested (Abad et al. 2009; Liefting et al. 2009b). The same bacterial pathogen was also associated with diseases of tomato and pepper in

Mexico (Munyaneza et al. 2009) and tobacco plants in Honduras (Aguilar et al. 2013). This bacterium has also been reported in a number of solanaceous weed species (Wen et al. 2009). In all of these cases, the bacterium was transmitted by the potato tomato psyllid (*Bactericera cockerelli*). This psyllid is endemic to America, but was accidentally introduced to New Zealand, where it was first reported to occur in 2006 (Thomas et al. 2011).

'Candidatus Liberibacter solanacearum' affecting apiaceous crops has been reported from Europe (Munyaneza et al. 2010a, b; Haapalainen 2014) and northern Africa (Tahzima et al. 2014). The bacterium is transmitted by the psyllids *Bactericera trigonica* (Alfaro-Fernandez et al. 2012b) and *Trioza apicalis* (Munyaneza et al. 2010a). The geographical distribution of plant-associated 'Ca. L.' species is presented in Map 3.

Map 3 Global occurrences of 'Candidatus Liberibacter' species



Source: Haapalainen (2014): 'Ca. L. africanus' (green), 'Ca. L. americanus' (black), 'Ca. L. asiaticus' (yellow), 'Ca. L. crescens' (black star), 'Ca. L. europaeus' (blue), 'Ca. L. solanacearum' (red).

'Candidatus Liberibacter' species are obligate parasites of plants and psyllids, and are only able to multiply inside their hosts. 'Candidatus Liberibacter' species enter the plant host as the psyllid feeds on phloem, and in turn enter the insect during feeding, making their way from the gut to the haemolymph and then to the salivary glands for transport to the next host (Cooper et al. 2014). In the plant host, 'Candidatus Liberibacter' species move with the phloem in the sieve tubes throughout the whole plant, including the roots.

'Candidatus Liberibacter' species and the psyllid vectors are suspected of adapting to new host plant species, following changes in vegetation and environmental factors (Haapalainen 2014). Native wild plants may have developed some resistance or tolerance to 'Ca. L.' species (Korsten et al. 1996; Albrecht & Bowman 2012). These wild plants carry low levels of bacteria without producing disease symptoms (Korsten et al. 1996; Albrecht & Bowman 2012). However, cultivated hosts, such as citrus, potato, carrot and celery plants, can be severely affected by 'Ca. L.' infections and may be relatively new hosts for these pathogens. Epidemics involving different plants, psyllids and 'Ca. L.' species are now emerging in different countries on several continents (Haapalainen 2014).

'*Candidatus Liberibacter asiaticus*' is the only species known to cope with relatively high temperatures (around 35 °C) (Bové et al. 1974; Lopes et al. 2009; Munyaneza et al. 2012c; Bové 2013). '*Candidatus Liberibacter africanus*', '*Ca. L. americanus*' and '*Ca. L. solanacearum*' are sensitive to temperatures above 32 °C (Bové et al. 1974; Lopes et al. 2009; Munyaneza et al. 2012a).

### 3.2 '*Candidatus Liberibacter solanacearum*'

'*Candidatus Liberibacter solanacearum*' was originally associated only with solanaceous crops and was distributed in Central America, North America, New Zealand (Nelson et al. 2011; Haapalainen 2014) and Norfolk Island (NIQS 2014).

Carrot plants showing symptoms of yellows disease were first observed in different regions of Spain in 1999 (Font et al. 1999). Yellows disease is characterised by small leaves with yellowing and reddening discolouration; proliferation of leaves and small roots; and deformation, reduction and early senescence of roots. Carrot fields in the Canary Islands were heavily infested with the psyllid, *Bactericera trigonica* (Font et al. 1999). Aster yellows phytoplasma and Stolbut phytoplasma were detected in the carrot plants from some of the affected areas. The symptoms observed in Spanish carrot growing regions resembled those caused by leafhopper transmitted phytoplasmas and spiroplasmas in carrots (Lee et al. 2003; Weintraub & Orenstein 2004; Lee et al. 2006; Duduk et al. 2008).

Mixed infections of phytoplasmas and liberibacters have been reported in potato and other solanaceous crops (Rubio-Covarrubias et al. 2006; Liefting et al. 2009b; Santos-Cervantes et al. 2010). In Mexico, phytoplasmas were detected in potato psyllids at a low infection rate, suggesting that liberibacters were the most likely cause of symptoms observed in the affected potato plants (Rubio-Covarrubias et al. 2006; Santos-Cervantes et al. 2010). Low levels of phytoplasma infection in carrots have been reported from Finland, suggesting that mixed infections of both phytoplasmas and liberibacters in crops affected by psyllids may be common (Munyaneza et al. 2011).

Symptoms observed in carrots in mainland Spain and the Canary Islands in 1999 (Font et al. 1999), were very similar to symptoms often observed in *Trioza apicalis*-'*Ca. L.*' affected carrots in Finland (Munyaneza et al. 2010a). Symptomatic plants and *B. trigonica* collected from carrot fields showing yellows disease in the Canary Islands were not tested for '*Ca. L.*' species, because this bacterium was not suspected of causing this disease at that time. However, recent studies reported the occurrence of '*Ca. L. solanacearum*' in both carrot crops and *B. trigonica* in the Canary Islands and other areas in Spain (Alfaro-Fernández et al. 2012a, b).

'*Candidatus Liberibacter solanacearum*' associated with carrots is distributed in Austria (EPPO Reporting Service 2015), France (Loiseau et al. 2014), Finland (Munyaneza et al. 2010a, b), Germany (Munyaneza et al. 2015), Morocco (Tahzima et al. 2014), Norway (Munyaneza et al. 2012b), Spain (Alfaro-Fernández et al. 2012a, b) and Sweden (Munyaneza et al. 2012a). '*Candidatus Liberibacter solanacearum*' is also reported to occur on celery in Austria (EPPO Reporting Service 2015) and Spain (EPPO 2012; Teresani et al. 2014b); and parsnip in Spain (Cambra et al. 2015).

### 3.2.1 Haplotypes of ‘*Candidatus Liberibacter solanacearum*’

Based on molecular studies isolates of ‘*Ca. L. solanacearum*’ from different geographical areas have been characterised into five haplotypes (Nelson et al. 2011; 2012). ‘*Candidatus Liberibacter solanacearum*’ isolates from North and Central America represent haplotypes A and B infect solanaceous plants (Hansen et al. 2008). ‘*Candidatus Liberibacter solanacearum*’ isolates associated with carrot in Northern Europe represent haplotype C (Nelson et al. 2011; 2013) and ‘*Ca. L. solanacearum*’ isolates from carrot and celery plants in Spain and Morocco represent haplotypes D and E (Nelson et al. 2013; Tahzima et al. 2014; Teresani et al. 2014b). The distribution, vectors and hosts of known haplotypes of ‘*Ca. L. solanacearum*’ are summarized in Table 8.

**Table 8: ‘*Candidatus Liberibacter solanacearum*’ haplotypes, vectors, natural hosts and distribution**

‘ <i>Ca. L. solanacearum</i> ’	Psyllid host/vector	Natural host plant	Distribution*
Haplotype A	<i>Bactericera cockerelli</i>	Solanaceae family	North and Central America, New Zealand, Norfolk Island
Haplotype B	<i>Bactericera cockerelli</i>	Solanaceae family	North and Central America
Haplotype C	<i>Trioza apicalis</i>	Apiaceae family (carrot)	Finland, Germany, Norway, Sweden
Haplotype D	<i>Bactericera trigonica</i>	Apiaceae family (carrot)	Spain (mainland and Canary Islands), Morocco
Haplotype E	<i>Bactericera trigonica</i>	Apiaceae family (carrot and celery)	Spain (mainland), France, Morocco

\* This table only lists countries where the ‘*Ca. L. solanacearum*’ haplotype has been confirmed. For example, this bacterium is reported to occur in carrot and celery in Austria; however, the associated haplotype/s are unknown.

Haplotype A has been found primarily from Honduras and Guatemala through to western Mexico, Arizona and California, and in New Zealand (Nelson et al. 2011). Haplotype B is currently found from eastern Mexico, northwards through Texas to south central Washington (Nelson et al. 2011). These haplotypes show some range overlap in Texas, Kansas and Nebraska (Nelson et al. 2011).

Haplotype C was first described in carrots (Nelson et al. 2011) and is present in Finland, Norway, Sweden and Germany (Nelson et al. 2012). Haplotype D is associated with carrots (Nelson et al. 2012) and is found in Spain, the Canary Islands and Morocco and is likely to be present in France (Teresani et al. 2015). Haplotype E was described in Spain associated with both carrot and celery crops (Teresani et al. 2014b) and is also in France and Morocco (Teresani et al. 2015).

### 3.2.2 Symptoms of ‘*Candidatus Liberibacter solanacearum*’

‘*Candidatus Liberibacter*’ species are limited to the phloem; therefore, once a plant has become infected the bacterium can move throughout the plant. Symptoms caused by ‘*Ca. L.*’ species are not constant over time or between locations and they vary with season, host, pathogen species and environmental conditions. For example, potato and tomato plants infected with ‘*Ca. L. solanacearum*’ can become severely diseased, whereas pepper and eggplant do not develop severe symptoms (Lin & Gudmestad 2013).

'*Candidatus Liberibacter solanacearum*' in potato plants is often unevenly spread throughout the plant; however, in carrot it becomes systemic (Nissinen et al. 2014). In celery plants, the '*Ca. L. solanacearum*' haplotype E causes stem proliferation and curling symptoms (Teresani et al. 2014b). Shoot proliferation is also seen in infected carrots in Spain and Morocco (Bertolini et al. 2014; Tahzima et al. 2014), suggesting differences in the bacteria-plant interaction when compared with the haplotype C, which does not induce shoot proliferation but causes discolouration of the carrot leaves.

### Symptoms caused by '*Ca. L. solanacearum*' in carrot

Symptoms caused by '*Ca. L. solanacearum*' in carrot plants include leaf yellowing, bronze or red leaf discolouration, reduced size of the main root and lateral root proliferation (Munyanenza et al. 2011; Figure 1B). Other symptoms include stunting and proliferation of dwarfed shoots, bushy tops and a dense hairy growth of secondary roots (Loiseau et al. 2014). The number of leaves showing discolouration symptoms and the root weight reduction correlated with a high titre of '*Ca. L. solanacearum*' in the plant (Nissinen et al. 2014). Infected plants show collapsed phloem cells and phloem tubes are densely colonised by bacteria (Figure 1C).

**Figure 1: Symptoms caused by '*Ca. L. solanacearum*' in carrot: (A) healthy carrot; (B) infected carrot showing discoloured leaves and reduced root volume; and (C) bacterial cells in a sieve tube element of an infected carrot root**



Source: Haapsalainen (2014).

### Symptoms caused by '*Ca. L. solanacearum*' in celery

In 2008, in Villena (Alicante, Spain), celery plants were observed showing an abnormal amount of shoots, curling of stems and yellowing (Figure 2A to D). These vegetative disorders were observed in a celery crop grown in close proximity to carrot plots showing a high prevalence of '*Ca. L. solanacearum*' infected plants. Molecular studies confirmed that celery plants were infected with '*Ca. L. solanacearum*' (Teresani et al. 2014b).



**Figure 2 Symptoms caused by '*Ca. L. solanacearum*' in celery: (A) infected celery (left) healthy celery (right); (B) infected celery showing abnormal shoot proliferation; (C) curling of stems; and (D) mild symptoms in plant**



Source: Teresani et al. (2014b).

### 3.2.3 Transmission of '*Candidatus Liberibacter solanacearum*'

'*Candidatus Liberibacter solanacearum*' is transmitted through vegetative propagation and naturally by several psyllid species (Teresani et al. 2014b). Recently, seed transmission has been proven in carrot (Bertolini et al. 2015). Even though the pathogens are bacteria, they are not known to be mechanically transmitted or spread by wind or rain.

#### Psyllid transmission

Psyllids are pests in their own right in addition to being vectors of '*Ca. L.*' species (Nehlin et al. 1994; Nissinen et al. 2007). Psyllids feed on plants by sucking the phloem through their stylet (Hodkinson 2009; Ouvrard 2015). The total number of eggs laid by a mated female can be from one hundred to one thousand (Hodkinson 2009) and can have several generations per year. Thus, psyllid populations can grow very rapidly if no control measures are taken. The leaves of psyllid-infested plants appear chlorotic or discoloured, and galled, and a heavy infestation can lead to premature senescence and plant death. The psyllid-feeding symptoms in plants are referred to as psyllid yellows (Sengoda et al. 2010).

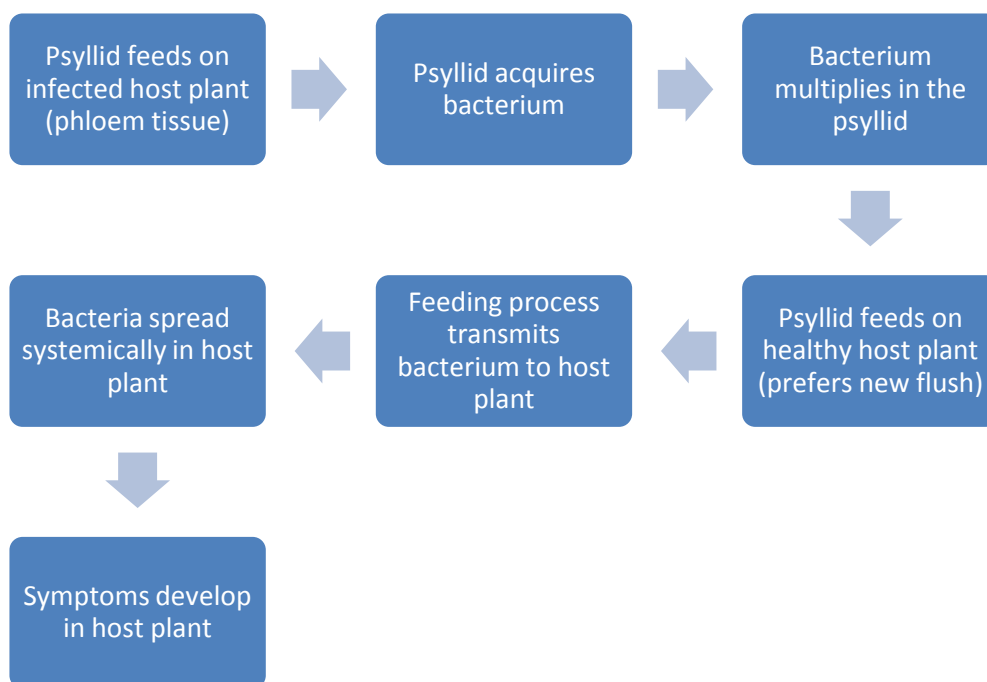


Psyllids acquire '*Ca. L.*' species through feeding on infected hosts (Bové 2006; Gottwald et al. 2007). When a psyllid feeds on the phloem of a '*Ca. L.*'-infected plant, the bacteria can be ingested by the psyllid.

'*Candidatus Liberibacter solanacearum*' associated with solanaceous crops have been detected in the psyllid *B. cockerelli* (Nelson et al. 2011). '*Candidatus Liberibacter solanacearum*' associated with apiaceous crops have been detected in *B. nigricornis*, *B. tremblayi*, *B. trigonica* and *T. apicalis* (Alfaro-Fernández et al. 2012b; Nelson et al. 2012; Teresani et al. 2014b). Of these psyllids, transmission has only been demonstrated in *Trioza apicalis* (Munyaneza et al. 2010a) and *Bactericera trigonica* (Alfaro-Fernández et al. 2012b).

*Bactericera trigonica*, *B. tremblayi* and *B. nigricornis* have been detected in carrot and celery crops in continental Spain. '*Candidatus Liberibacter solanacearum*' was detected in all these psyllids; therefore, these psyllids are potential vectors of the bacterium (Teresani et al. 2015). Once the psyllids have acquired the bacterium from an infected host, they maintain the ability to transmit the bacterium in a persistent manner (Teresani et al. 2015). Transmission of the bacteria by psyllid vectors is presented in Figure 3.

**Figure 3** Transmission of '*Candidatus Liberibacter solanacearum*' through psyllids



The psyllid acquires the bacterium within two hours of colonising the plant (Munyaneza 2010); the bacterium then multiplies in the psyllid and transmission happens within three days (Nissinen et al. 2014). The symptoms of leaf discolouration in carrot become visible within 1–1.5 months (Nissinen et al. 2007; 2012).

'*Candidatus Liberibacter solanacearum*' titre in carrots is correlated with the bacterial titre in the psyllids. High '*Ca. L. solanacearum*' titre in the plants significantly reduces the root weight, but does not affect the number of curled leaves. The subsequent leaf discolouration symptom is thought to be caused by the multiplication of the bacteria (Nissinen et al. 2014).

## Seed Transmission

'*Ca. L. solanacearum*' associated with apiaceous crops have been detected in carrot seeds and seed transmission has been demonstrated (Bertolini et al. 2015).

### 3.2.4 Psyllid vectors and carriers of '*Candidatus Liberibacter solanacearum*' in apiaceous crops

'*Candidatus Liberibacter solanacearum*' is transmitted by different psyllid species (Teresani et al. 2015). These psyllid vectors are damaging to the host crops (Nehlin et al. 1994; Nissinen et al. 2007). *Bactericera trigonica* and *T. apicalis* are efficient vectors of '*Ca. L. solanacearum*' (Nelson et al. 2011; Alfaro-Fernández et al. 2012b; Nelson et al. 2012; Teresani et al. 2014b). '*Candidatus Liberibacter solanacearum*' has also been detected in *B. tremblayi* and *B. nigricornis* collected from carrot and celery fields in Spain (Teresani et al. 2015).

#### *Trioza apicalis*

The origin of *Trioza apicalis* is unknown; however, this psyllid has existed for at least several thousand years in temperate and northern Eurasia (Láska 2011). Over 160 years ago, *T. apicalis* was described from Germany (Foerster 1848, cited in Láska 2011). The psyllid slowly expanded its range and reached continental Denmark in about 1912 (Rostrup 1921, cited in Láska 2011). The psyllid then expanded its range from Denmark to neighbouring countries including Latvia, Sweden and Norway. *Trioza apicalis* was reported in Czechoslovakia in 1936 (Baudyš 1936, cited in Láska 2011). *Trioza apicalis* is associated with carrot in Scandinavia, Finland and other parts of northern and central Europe, although it occurs in wider areas of Eurasia from Great Britain to Mongolia (Hodkinson 1984; Láska 2011; Map 4).

Map 4 Distribution of *Trioza apicalis*



Source: Ouvrard (2015).

*Trioza apicalis* has only one generation per year (Kristoffersen & Anderbrant 2007). The adult females of *Trioza apicalis* lay eggs in the leaf tissue (Figure 4B) during summer. Embryonic

development takes 12 to 14 days on average under field conditions (Láska 1974). The total development time (including eggs) under field conditions (at a mean temperature of 17 °C) takes a median of 54 days (Láska 1974).

**Figure 4** *Trioza apicalis*: (A) adult; (B) female on a carrot leaf; (C) small elongated eggs; and (D) nymphs



Source: Meadow (2010); Munyaneza et al. (2010b).

The feeding activity of *Trioza apicalis* causes curling of the youngest leaves (Figure 5). The first record of curling of carrot leaves dates back to 1896; however, the cause of this damage was identified in 1908 as *T. viridula* (Rostrup 1921, cited in Láska 2011). Later on, similar damage on carrot was reported on the Danish island of Sjælland (Rostrup 1921, cited in Láska 2011). Subsequently, damage in carrot was reported from Germany (Krumrey & Wendland 1973) and Switzerland (Burckhardt & Freuler 2000; Fischer & Terretaz 2002).

**Figure 5** Leaf curling caused by the carrot psyllid



Source: Munyaneza et al. (2010b).

In addition to leaf curling, it was recognised that *T. apicalis* feeding damage includes yellowish, bronze, and purplish discolouration of leaves; stunting of the shoots and roots; and proliferation of secondary roots (Markkula et al. 1976; Nehlin et al. 1994; Nissinen et al. 2007; Figure 6).

**Figure 6 Symptoms developed on carrot after psyllid exposure: (A) healthy leaf; (B) leaf showing discolouration; (C) damage without discolouration; and (D) proliferation of secondary roots**



Source: Munyaneza et al. (2010b).

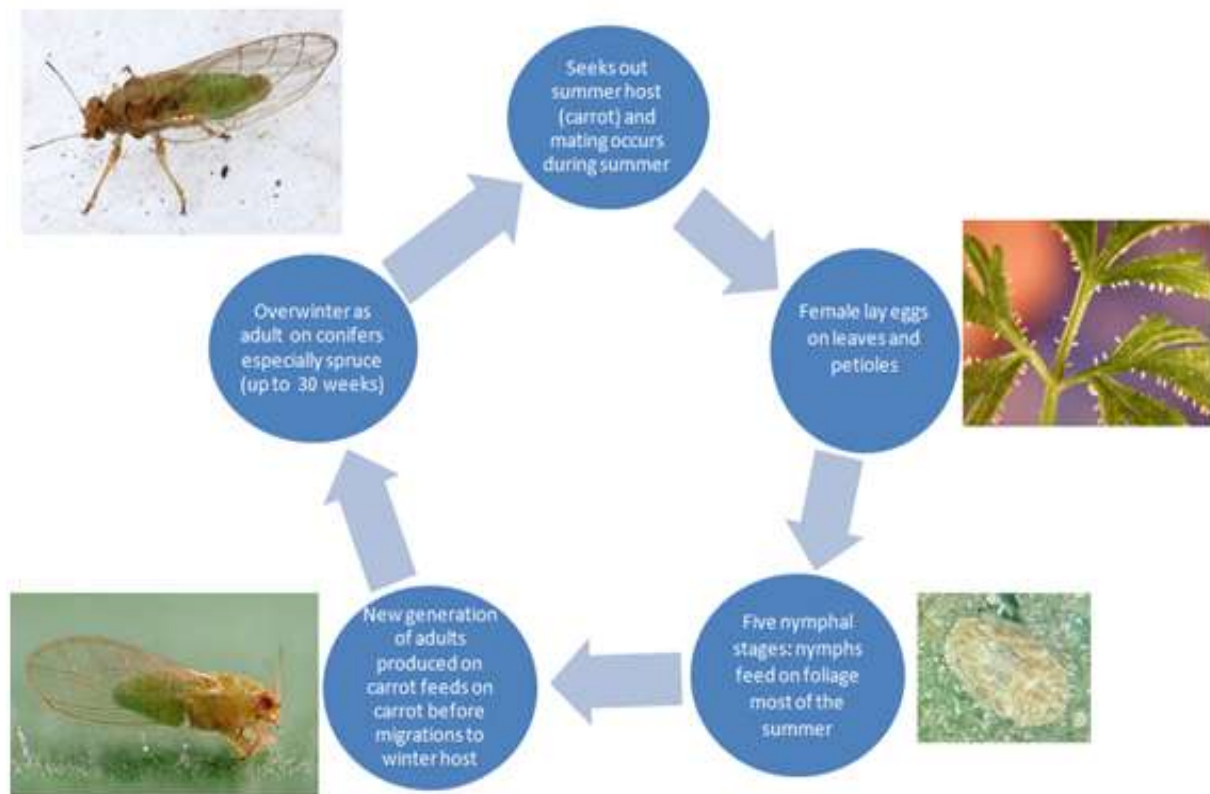
Symptoms caused in carrot were considered to be a result of toxins injected by the psyllid during feeding activities (Markkula et al. 1976; Nehlin et al. 1994). However, no toxin was identified from the extractions of psyllid salivary glands and salivary secretions (Markkula & Laurema 1971). Further studies demonstrated that the short feeding period of *T. apicalis* results in the development of severe symptoms in carrots that usually appear one month after insect removal (Nissinen et al. 2007). These studies suggested that plant pathogens may be involved in this *T. apicalis*-induced disorder (Nissinen et al. 2007). Subsequent studies discovered that '*Ca. L. solanacearum*' is associated with both the psyllid and the carrot plant (Munyaneza et al. 2010a, b). Leaf discolouration and root size reduction are correlated with the colonisation of the carrot phloem vessels by the bacterium. The bacteria colonises the phloem vessels systemically and proliferate to a high density, filling the sieve cells (Haapalainen et al. 2014).

The psyllid lifecycle begins with mating between fertile adults on summer hosts (Kristoffersen & Anderbrant 2007). The adult females lay eggs on the host plant leaves. After egg hatching, nymphs develop through five instar stages, and then adults (Figure 7). The time needed for development from eggs to mature adults varies in length depending on temperature, but is usually about one month (Hodkinson 2009). The reproductive biology of psyllids is closely tied to the availability of the new leaf flush for egg laying and subsequent development of nymphs. As these leaves mature, they become unsuitable for psyllid development and the psyllids seek out new breeding sites (Hodkinson 2009). Depending on the season and climate conditions, the



newly emerged adults either fly to new summer host plants to reproduce, or migrate to overwintering hosts (Kristoffersen & Anderbrant 2007). The period spent overwintering on shelter plants usually matches the period when the summer host is dormant or unfavourable for psyllid development. The adult carrot psyllids leave their overwintering host in late spring or early summer, usually at the time when the main summer host is emerging. Photoperiod plays a role in the migration between winter and summer hosts (Valterová et al. 1997). In addition, changes in the concentration of secondary metabolites in the winter host could affect the timing of the migration of carrot psyllids (Nissinen et al. 2008).

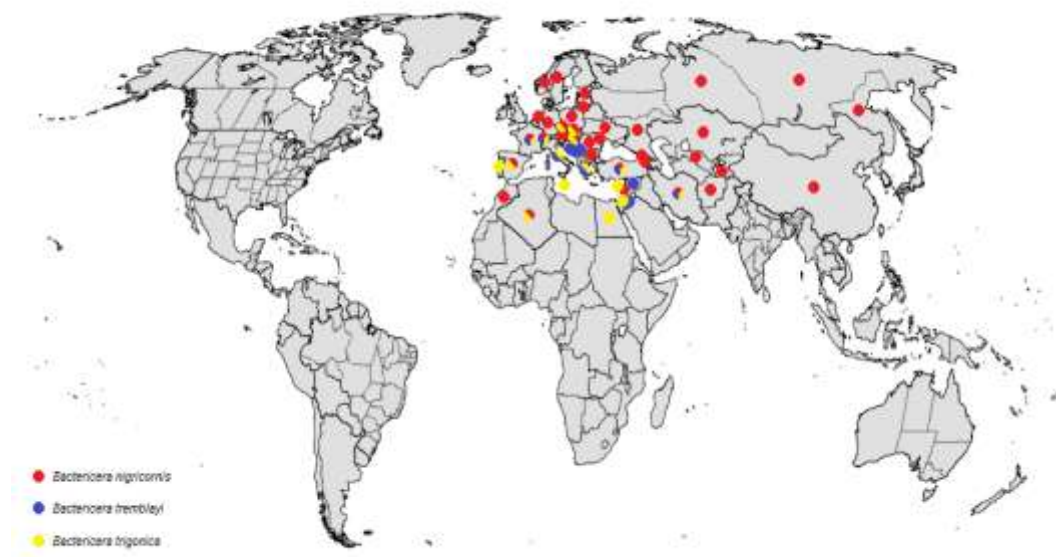
Figure 7 Life cycle of *Trioza apicalis*



Host selection by insects is based on various stimuli released by the host plant, and consists of a sequence of behavioural responses (Visser 1986; Stadler 1992). Different types of compounds released from carrots have been identified (Buttery et al. 1968; Simon et al. 1980). For example, host selection in *T. apicalis* seems to be connected to the high release of sabinene and apinene (Nehlin et al. 1996).

#### **'*Bactericera nigricornis* group'**

*Bactericera nigricornis*, *B. tremblayi* and *B. trigonica* are morphologically similar psyllid species that belong to the '*Bactericera nigricornis* group' (Hodkinson 1981). Members of this group are polyphagous, show overlapping areas of distribution and are widely distributed in the Mediterranean region (Alfaro-Fernández et al. 2012b; Ouvrard & Burckhardt 2012; Haapalainen 2014; Teserani et al. 2015; Map 5).

Map 5 Distribution of '*Bactericera nigricornis* group'

The species within the '*B. nigricornis* group' are multivoltine (Hodkinson 2009), overwinter as adults and feed on a variety of herbaceous plants, including beet, cabbage, carrot, onion, parsley and potato (Burckhardt & Lauterer 1997; Lauterer 1991). *Bactericera nigricornis*, *B. tremblayi* and *B. trigonica* are found in carrot and celery fields in Spain (Font et al. 2010; Alfaro-Fernández et al. 2012a; Teresani et al. 2015). '*Ca. L. solanacearum*' has been detected in these psyllid species, therefore; these psyllids are potential vectors of the bacterium (Teresani et al. 2015). It is suspected that more psyllid species may be vectors of '*Ca. L. solanacearum*'. Overlapping host ranges would provide ample opportunity for other members of the '*B. nigricornis* group' that feed on carrot to acquire the '*Ca. L. solanacearum*' pathogen.

'*Candidatus Liberibacter solanacearum*' associated with apiaceous crops has been detected in a potato plant growing in a carrot field, which was heavily infested with *T. apicalis* (Nissinen et al. 2014; FERA 2014). However, it is not clear whether carrot '*Ca. L. solanacearum*' (haplotypes C, D & E) can produce systemic infections in potato.

### ***Bactericera nigricornis***

The origin of *B. nigricornis* (synonym *Trioza nigricornis*) is unknown, but it was described from Germany (Forster 1848, cited in Hodkinson 1981) and has subsequently been recorded throughout Europe, Asia Minor, North Africa and from as far east as Siberia (Hodkinson 1981). Earlier studies suggested that *B. nigricornis* is highly polyphagous, feeding and developing on carrot, parsley, potato, beet, brassicas, onion and radish in addition to a variety of weed species in a number of families (Hodkinson 1981). Adults are also reported to overwinter on conifers (Reuter 1908, cited in Hodkinson 1981; Figure 8A). In Sweden, *B. nigricornis* was reported to breed on *Brassica* species and adults to occur, but not breed, on carrot (Lundblad 1929, cited in Hodkinson 1981). In contrast, in Germany this psyllid was reported to breed on carrot and parsley, while beetroot and Jimson weed (*Datura stramonium*) were alternative hosts (Bey 1931, cited in Hodkinson 1981). Later on, potato and *Brassica* species were listed as the main

hosts of this psyllid in Germany (Heinze & Profit 1939, cited in Hodkinson 1981), Sweden and the Netherlands (Ossiannilsson 1943 and Gravestein 1949, cited in Hodkinson 1981).

**Figure 8** *Bactericera nigricornis*: (A) adult; (B) small elongated eggs; (C) eggs on stalk; (D) nymphs; and (E) adult



Source: Ouvrard (2015).

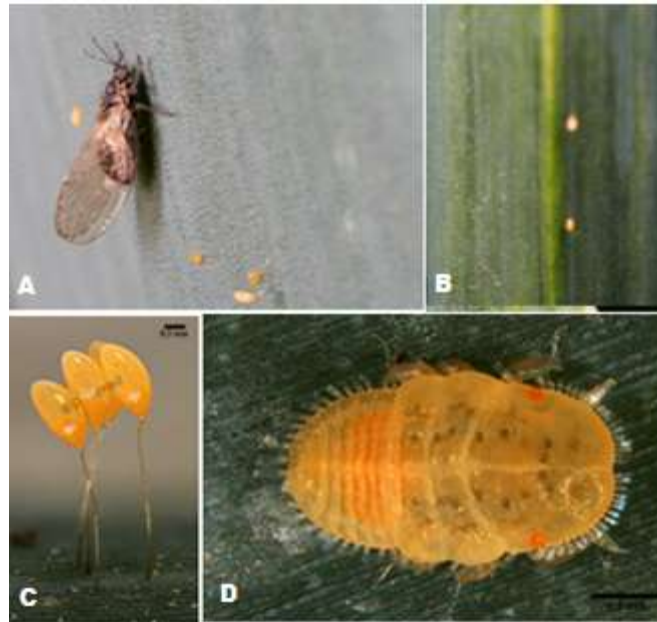
*Bactericera nigricornis* is a major pest of potatoes in Iran (Fathi 2011). Females lay eggs on the lower surface of potato leaves. After hatching, the nymphs feed on plant sap and excrete large amounts of honeydew (Fathi & Nouri-Ganbalani 2008). Field observations in Iran indicate that in potato fields infested with *B. nigricornis*, yield is decreased, and a striped pattern of necrosis develops in the tuber cross-section (Fathi 2011). These symptoms resemble those that develop in tubers of potato infested with *B. cockerelli* (Fathi & Nouri-Ganbalani 2008). However, currently there is no information on the presence of 'Ca. Liberibacter solanacearum' and *B. cockerelli* in Iran. *Bactericera nigricornis* may possibly transmit Zebra Chip disease in infested potatoes, but no study has investigated this possibility in Iran (Fathi 2011).

### ***Bactericera tremblayi***

*Bactericera tremblayi* was described on onion, in Italy (Wagner 1961; Tremblay 1965a, b). However, the psyllid was also reported to breed on *Brassica* species, *Capsella burso-pastori*, *Capsicum* species, *Chenopodium* species and *Stellaria media* (Tremblay 1965a, b). Oviposition did not occur on small, hairy potato plants but some eggs (Figure 9B) and nymphs (Figure 9D) were found on a larger potato plant. Therefore, this psyllid appears to be polyphagous and share several common host-plants with *B. nigricornis*. The range of this species has subsequently been extended to include Turkey (Klimaszewski & Lodos 1979) and possibly Syria (Klimaszewski 1968). Some authors have continued to apply the name *B. nigricornis* to a species breeding on wild onions (*Allium* species) in Mongolia and Caucasus (Loginova 1968; 1970).



**Figure 9** *Bactericera tremblayi*: (A) adult; (B) small elongated eggs; (C) eggs on stalk; and (D) nymphs



Source: Ouvrard (2012; 2015).

In France, high populations of *B. tremblayi* have been reported to cause new symptoms on *Allium porri* (Ouvrard 2012). Symptoms include longitudinal yellow stripes on the cylinder, bursting of bundled leaf sheaths and root growth between the burst sheaths. The aerial tip of the green leaves wither; the colour changes from bluish-green to dark shiny green and eventually the plant may die (Ouvrard 2012). These symptoms suggest that the psyllid may be vectoring a pathogen; however, the pathogen has not been identified (Ouvrard 2012). 'Candidatus Liberibacter solanacearum' has been detected in *B. tremblayi* collected from carrot and celery fields in Spain (Teresani et al. 2015).

### ***Bactericera trigonica***

*Bactericera trigonica* was described from carrot (Portugal), nasturtium (Iran) and other vegetables (Cyprus) (Hodkinson 1981). *Bactericera trigonica* feeds on carrots and related plants, and the adults overwinter in evergreen shrubs (Hodkinson 2009). *Bactericera trigonica* has two or three generations per year (Hodkinson 2009).

*Bactericera trigonica* has been reported from Algeria, Cyprus, Czech Republic, Egypt, France, Greece, Hungary, Iran, Israel, Italy, Malta, Portugal, Slovakia, Spain, Switzerland and Turkey (Ouvrard 2015).

A large population of *B. trigonica* was noted in carrot fields showing symptoms of curling; yellow, bronze, and purple discolouration of leaves; stunting of shoots and tap roots; and proliferation of secondary roots (Alfaro-Fernández et al. 2012a, b). 'Candidatus Liberibacter solanacearum' was detected in carrots and *B. trigonica* (Alfaro-Fernández et al. 2012b). This was the first report of this plant pathogen associated with *B. trigonica* (Alfaro-Fernández et al. 2012b).

## 4 Pest risk assessment

The pest risk assessment provides technical justification for identifying quarantine pests and for establishing phytosanitary import requirements. This pathogen-based risk assessment was initiated due to the identification of '*Ca. L. solanacearum*' on apiaceous hosts and new evidence of it being seed-borne and seed transmitted in carrot (Bertolini et al. 2014). '*Candidatus Liberibacter solanacearum*' is identified as a quarantine pathogen in the pest categorisation process because it:

- is not present in Australia;
- has the potential to be associated with the pathways of seeds (carrot) and tissue cultures (carrot, celery and parsnip);
- has the potential to establish and spread in Australia; and
- has the potential for significant economic consequences in Australia.

### 4.1 '*Candidatus Liberibacter solanacearum*'

Three haplotypes of '*Ca. L. solanacearum*' (C, D & E) have been identified as associated with apiaceous crops (carrot, celery and parsnip). Therefore, this risk assessment focuses on apiaceous propagative material (seeds and tissue cultures) as potential pathways for the introduction of '*Ca. L. solanacearum*' haplotypes associated with apiaceous crops.

The probability of entry has been considered individually for each pathway, as the pathway by which '*Ca. L. solanacearum*' haplotypes might enter Australia may have a significant effect on the unrestricted risk estimate.

The probability of establishment and spread, and the assessment of potential consequences, are considered to be independent of the pathway of entry. Instead, they are influenced by post-border issues, such as the susceptibility of hosts, the availability of hosts and the suitability of the environment in Australia for '*Ca. L. solanacearum*' (haplotypes C, D & E). Therefore, the probability of establishment and spread and the economic consequences of '*Ca. L. solanacearum*' have been assessed only once.

#### 4.1.1 Likelihood of entry

The likelihood of entry is divided for assessment purposes into the likelihood of importation (the likelihood that the '*Ca. L. solanacearum*' will arrive when seeds for sowing (carrot) and tissue cultures (carrot, celery and parsnip) are imported) and the likelihood of distribution (the likelihood that '*Ca. L. solanacearum*' associated with seeds for sowing (carrot) and tissue cultures (carrot, celery and parsnip) will be viable and be transferred to suitable host).

##### **Pathway One: Seeds for sowing (carrot)**

Seed pathogens have evolved many different types of associations with their hosts. These associations span a continuum of relationships that range from passive hitchhiking on seed coats to infecting embryonic tissue (Elmer 2001).

### **Likelihood of importation**

The likelihood that the '*Ca. L. solanacearum*' will arrive in Australia with trade in carrot seeds for sowing is **HIGH**.

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

- '*Ca. L. solanacearum*' is reported as seed-borne in carrot (Bertolini et al. 2015); therefore, this bacterium is associated with the carrot seed pathway.
- Global trade and the associated movement of carrot seeds across borders may have introduced '*Ca. L. solanacearum*' into new areas (Bertolini et al. 2015). Therefore, the movement of infected carrot seeds may be a significant pathway for the introduction of this pathogen into new areas.
- '*Ca. L. solanacearum*' was detected in carrot seed lots from 2010 to 2014 (Bertolini et al. 2015), indicating that the bacterium is able to persist in seeds and is therefore associated with the pathway.

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

- '*Ca. L. solanacearum*' is very likely to survive during transport and storage since the primary conditions for survival are fulfilled by the presence of the live host material and associated environmental conditions. Carrot seeds are packaged and shipped to areas conducive to their survival. The handling of carrot seeds is unlikely to be detrimental to the survival of this pathogen.
- Most seed-borne pathogens infect and use seeds as a vehicle for transport and survival (Elmer 2001). Seed associations can provide long-term survival for pathogens. Survival is highly unlikely to be diminished during transport or storage (Elmer 2001).
- Transport and storage of propagative material is done at low temperatures and these conditions are not expected to affect the viability of the '*Ca. L. solanacearum*'. Therefore, '*Ca. L. solanacearum*' is likely to survive transport and storage.
- The transportation of carrot seeds from the country of origin to Australia may take several days. There is no reason to suspect that transportation conditions would affect the viability of '*Ca. L. solanacearum*' within the seed.
- '*Ca. L. solanacearum*' is found within carrot seed and is unlikely to be dislodged during standard harvesting, handling and shipping operations. Therefore, '*Ca. L. solanacearum*' associated with carrot seeds are likely to survive during transport and storage.

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

- Currently, there are few effective control strategies for the protection of host crops against natural infections of '*Ca. L. solanacearum*'.
- '*Candidatus Liberibacter*' species are typically managed by chemical control of vector populations and the removal of the inoculum source. Crops may also be cultivated under insect-proof facilities to exclude vectors.
- The use of '*Ca. L. solanacearum*'-free carrot seeds and management of psyllid vectors are critical factors in managing '*Ca. L. solanacearum*' in carrot.

- Plant protection measures are mostly taken against the psyllids. Measures against psyllids have been implemented everywhere that the psyllid and the psyllid/bacterium combination are present, in order to keep damage under a threshold level. Treatments against the insect vector reduce the incidence of damage but do not completely suppress the bacterium. In some years in northern Europe, carrots cannot be grown without the application of insecticides. For example, insecticide treatment is an economic necessity in Norway, Sweden, Denmark, Latvia and Switzerland (Láska 2011).

#### **Likelihood of distribution (transfer to a susceptible host)**

The likelihood that '*Ca. L. solanacearum*' will be distributed within Australia in a viable state with imported carrot seeds for sowing and be transferred from the resulting carrot plants to a suitable host is **LOW**.

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

- '*Ca. L. solanacearum*' is seed-borne and seed transmissible in carrot (Bertolini et al. 2015). Therefore, '*Ca. L. solanacearum*' arriving in Australia with imported infected carrot seeds for sowing is already present within a suitable host that will be used for propagation.
- Although '*Ca. L. solanacearum*' is unable to move independently from imported seeds to a suitable host, the bacterium has been shown to be transmitted from seed to the resultant seedling (Bertolini et al. 2015). The bacterium is likely to survive as long as its host plant is present. In the case of annual crops, such as carrot for consumption that are harvested before seed are produced, the bacterium will depend on a vector to transfer it to a reservoir host for survival during the cropless period and infection of the next generation of carrot seed.
- The transfer of '*Ca. L. solanacearum*' from an infected seedling to other suitable hosts or carrot plants within a field would require a vector. In the absence of a vector, '*Ca. L. solanacearum*' could only persist through multiple generations of seed to seedling transmission.
- Carrot seeds are imported specifically for the purpose of propagation and can be a significant investment for importers. Infected carrot seeds are therefore likely to be grown directly in suitable habitats at multiple locations throughout Australia. The distribution of infected carrot seeds commercially will assist in the distribution of '*Ca. L. solanacearum*' within Australia.
- '*Ca. L. solanacearum*' haplotypes C, D and E are host specific to carrot and celery (Munyaneza et al. 2010a; Teresani et al. 2014b). Carrot and celery crops are widely distributed throughout Australia with many residential and semi-rural properties in the metropolitan area growing vegetables in the backyard. However, in the absence of psyllid vectors, the bacterium is unlikely to move to these available hosts.
- '*Ca. L. solanacearum*' has been detected in three species of *Bactericera* (*B. nigricornis*, *B. tremblayi* and *B. trigonica*) and *T. apicalis*. However, only *B. trigonica* and *T. apicalis* are known to transmit '*Ca. L. solanacearum*' naturally (Haapalainen 2014). This vector specificity suggests it is unlikely that Australian native psyllids would be able to transmit '*Ca. L. solanacearum*'.
- In carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot seeds.

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

- The distribution of carrot seeds for sowing would be for commercial and retail distribution to multiple destinations throughout Australia. Carrot seeds for sowing would also be distributed throughout Australia for propagation.
- The distribution of infected seeds commercially through seed companies may facilitate the distribution of '*Ca. L. solanacearum*' in Australia. Asymptomatic plants that develop from infected seeds may also be overlooked and sold to commercial users and households.
- '*Ca. L. solanacearum*' is likely to survive transportation and storage within Australia. Carrot seeds are likely to be transported, stored and maintained in appropriate conditions. Thus, transport and storage conditions within Australia are unlikely to have any impact on the survival of '*Ca. L. solanacearum*' in imported seeds.
- Association with carrot seeds provides the opportunity for '*Ca. L. solanacearum*' to enter Australia. Its ability to survive on, or in, propagative material acts to ensure the viability of the bacterium on route to, and during distribution across, Australia.

**Risks from by-products and waste**

- The intended use of carrot seeds is for propagation, and all imported seeds would be grown under ideal conditions. Carrot seeds that do not survive transportation and storage may not be used for sowing. Therefore, waste material may be generated. As the bacterium can infect seeds, any such material that is discarded may contain the bacterium.
- If a seed does germinate and seed to seedling transmission occurs, an infected plant will establish. The transfer of '*Ca. L. solanacearum*' from an infected seedling established at a waste depot or in the backyard, to a host would require a vector.
- The transfer of '*Ca. L. solanacearum*' from an established seedling at a waste depot or in the backyard, to a new host would then require a vector.
- '*Ca. L. solanacearum*' has been detected in three species of *Bactericera* (*B. nigricornis*, *B. tremblayi* and *B. trigonica*) and *T. apicalis*. However, only *B. trigonica* and *T. apicalis* are known to transmit '*Ca. L. solanacearum*' naturally (Haapalainen 2014). This vector specificity suggests it is unlikely that Australian native psyllids would be able to transmit '*Ca. L. solanacearum*'.
- Since '*Ca. L. solanacearum*' requires a vector, it is highly likely that an infected plant, which develops in an isolated area where the psyllid vector is not known to occur, will represent an isolated infection.
- In carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot seeds. In the absence of a suitable vector, it is likely that these cases will not lead to long term establishment, but they could lead to transient '*Ca. L. solanacearum*' populations.

**Pathway two: Tissue cultures (carrot, celery and parsnip)**

'*Candidatus Liberibacter*' species infect phloem; therefore, once a plant has become infected, the bacterium can move throughout the plant. Consequently, carrot, celery and parsnip tissue cultures provide a pathway for '*Ca. L. solanacearum*'.

### **Likelihood of importation**

The likelihood that '*Ca. L. solanacearum*' will arrive in Australia with trade in tissue cultures (carrot, celery and parsnip) for propagation is **HIGH**.

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

- '*Ca. L. solanacearum*' is distributed systemically in all parts of the infected plant, and is found in the phloem of leaflets, petioles, stems, crowns and roots (Nissinen et al. 2014). Therefore, this bacterium is associated with the tissue culture pathway.
- Since '*Ca. L.*' species infect phloem, once a plant has become infected the bacterium can move throughout the plant. Global trade and the associated movement of carrot, celery and parsnip tissue cultures across borders may introduce '*Ca. L. solanacearum*' into new areas.
- '*Ca. L.*' species survive within the living phloem cells in the vascular system of host plants. The presence 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).
- Carrot and celery plants infected with '*Ca. L. solanacearum*' may be symptomless (Teresani et al. 2014b), which could also contribute to the introduction and spread of the bacterium in tissue cultures.

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

- '*Ca. L. solanacearum*' is very likely to survive during transport and storage since the primary conditions for survival are fulfilled by the presence of the live host material (tissue cultures) and associated environmental conditions. Tissue cultures are packaged and shipped to areas conducive to their survival. The handling of tissue cultures is unlikely to be detrimental to the survival of this pathogen.
- The transportation of tissue cultures from the country of origin to Australia may require several days. There is no reason to suspect that these conditions would affect the viability of '*Ca. L. solanacearum*' because the bacterium is present within live host tissue cultures.
- Transport and storage of propagative material is done at low temperatures and these conditions are not expected to affect the viability of '*Ca. L. solanacearum*'. Therefore, '*Ca. L. solanacearum*' is likely to survive transport and storage.

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

- Currently, there are few effective control strategies for the protection of host crops against natural infections of '*Ca. L. solanacearum*'.
- '*Candidatus Liberibacter*' species are typically managed by chemical control of vector populations and the removal of the inoculum source. Crops may also be cultivated under insect-proof facilities to exclude vectors.
- The use of '*Ca. L. solanacearum*'-free tissue cultures and management of psyllid vectors are critical factors in managing '*Ca. L. solanacearum*' in carrot and celery crops.
- Plant protection measures are mostly taken against the psyllids. Measures against psyllids have been implemented everywhere that the psyllid and the psyllid/bacterium combination are present, in order to keep damage under a threshold. Treatments against the insect

vectors reduce the incidence of damage but may not completely suppress the bacterium. In some years in northern Europe, carrots cannot be grown without the application of insecticides. For example, insecticide treatment is an economic necessity in Norway, Sweden, Denmark, Latvia and Switzerland (Láska 2011).

### **Likelihood of distribution (transfer to a susceptible host)**

The likelihood that '*Ca. L. solanacearum*' will be distributed within Australia in a viable state with imported tissue cultures (carrot, celery and parsnip) for propagation and be transferred from these tissue cultures to a suitable host is **LOW**.

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

- '*Ca. L. solanacearum*' arriving in Australia with imported infected tissue cultures is already present within a suitable host that will be used for propagation. In the absence of a vector, the bacterium is unlikely to move from infected seedlings to new host plants; infected plants will ultimately senesce and the bacterium may not persist in the environment.
- Tissue cultures are imported specifically for the purpose of propagation and can be a significant investment for importers. Infected tissue cultures are therefore likely to be grown directly into suitable habitats at multiple locations throughout Australia. The distribution of infected tissue cultures commercially will assist in the distribution of '*Ca. L. solanacearum*'.
- '*Ca. L. solanacearum*' haplotypes C, D and E are host specific to carrot and celery (Munyaneza et al. 2011; Teresani et al. 2014b). These host species are widely distributed throughout Australia with many residential and semi-rural properties in the metropolitan area growing vegetables/food plants in the backyard. However, in the absence of psyllid vectors, the bacterium is unlikely to move to these available hosts.
- '*Ca. L. solanacearum*' has been detected in three species of *Bactericera* (*B. nigricornis*, *B. tremblayi* and *B. trigonica*) and *T. apicalis*. However, only *B. trigonica* and *T. apicalis* are known to transmit '*Ca. L. solanacearum*' naturally (Haapalainen 2014). This vector specificity suggests it is unlikely that Australian native psyllids would be able to transmit '*Ca. L. solanacearum*'.
- In carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot tissue cultures. In the absence of a suitable vector, it is likely that these cases will not lead to long term establishment, but they could lead to transient '*Ca. L. solanacearum*' populations.

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

- The distribution of tissue cultures for propagation would be for commercial and retail distribution to multiple destinations throughout Australia. Therefore, the bacterium will also be distributed throughout Australia.
- Distribution of infected tissue cultures through nurseries may facilitate the distribution of '*Ca. L. solanacearum*' throughout Australia. Asymptomatic plants that develop from infected tissue cultures may be overlooked and sold to commercial users and households.
- Tissue cultures are likely to be transported, stored and maintained in appropriate conditions. Thus, transport and storage conditions within Australia are unlikely to have any adverse impact on the survival of '*Ca. L. solanacearum*' in imported tissue cultures.

- Association with carrot and celery tissue cultures provides the opportunity for ‘*Ca. L. solanacearum*’ to enter Australia. The ability of ‘*Ca. L. solanacearum*’ to survive within propagative material, acts to ensure its viability on route to and during distribution across Australia.

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

- The intended use of tissue cultures is for propagation, and all imported tissue cultures would be grown under ideal conditions. Tissue cultures that do not survive transportation and storage may not be used for propagation. Therefore, waste material may be generated. As the bacterium can infect the entire plant (Nissinen et al. 2014), any such material that is discarded may contain the bacterium.
- The transfer of ‘*Ca. L. solanacearum*’ from a tissue culture plantlet established at a waste depot or in the backyard, to a new host would then require a vector.
- ‘*Ca. L. solanacearum*’ has been detected in three species of *Bactericera* (*B. nigricornis*, *B. tremblayi* and *B. trigonica*) and *T. apicalis*. However, only *B. trigonica* and *T. apicalis* are known to transmit ‘*Ca. L. solanacearum*’ naturally (Haapalainen 2014). This vector specificity suggests it is unlikely that Australian native psyllids would be able to transmit ‘*Ca. L. solanacearum*’.
- Since ‘*Ca. L. solanacearum*’ requires a vector, it is highly likely that an infected plant, which develops in an isolated area where the psyllid vector is not known to occur, will represent an isolated infection.
- In carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot tissue cultures. In the absence of a suitable vector, it is likely that these cases will not lead to long term establishment, but they could lead to transient ‘*Ca. L. solanacearum*’ populations.

#### **Overall likelihood of entry (importation x distribution)**

The overall likelihood of entry ‘*Ca. L. solanacearum*’ is determined by combining the likelihood of importation with the likelihood of distribution using the matrix of rules for combining likelihoods (Table 3). The overall likelihood of entry on different pathways being assessed in this PRA is set out in Table 9.

**Table 9 Overall likelihood of entry of ‘*Candidatus Liberibacter solanacearum*’ on different pathways**

Pathway	Likelihood of importation	Likelihood of distribution	Overall likelihood of entry
Seeds for sowing	High	Low	Low
Tissue cultures	High	Low	Low

#### **4.1.2 Likelihood of establishment**

The likelihood that ‘*Ca. L. solanacearum*’, having entered on imported propagative material (seeds and tissue cultures), will establish within Australia based on a comparison of factors in the source and destination areas considered pertinent to its survival and reproduction is **MODERATE**.



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

- Association with the host propagative material will facilitate the establishment of '*Ca. L. solanacearum*', as the pathogen is already established within a suitable host. As host plant material is likely to be maintained in places with similar climates to the area of production, climatic conditions are expected to favour the pathogen's establishment.
- Seeds and tissue cultures are intended for ongoing propagation and are deliberately introduced, distributed and aided to establish. This material will enter and then be maintained in a suitable habitat, potentially in substantial numbers and for an indeterminate period. Therefore, the introduction and establishment of plants from imported seeds and tissue cultures in essence establishes those pathogens associated with the propagative material.
- Studies on carrot seeds have demonstrated that the bacterium is seed-borne and is transmitted from seed to seedling (Bertolini et al. 2014). Therefore, the introduction and establishment of plants from imported seeds allows the establishment of '*Ca. L. solanacearum*' associated with the propagative material.
- Carrot, celery and parsnip are widely cultivated throughout Australia, with many residential and semi-rural properties in the metropolitan areas growing vegetables—including carrot, celery and parsnip—in the backyard.
- The latent period of infection before visible symptoms appear may result in non-detection of '*Ca. L. solanacearum*'; therefore, '*Ca. L. solanacearum*' will have ample time to establish in new areas. In seed to seedling transmission studies, visible symptoms may appear after 60 days (Bertolini et al. 2015). In psyllid transmission studies, symptoms become visible on carrots one to two months after psyllid feeding (Haapalainen et al. 2014).
- If '*Ca. L. solanacearum*' is introduced through trade in seeds (carrot) or tissue cultures (carrot, celery, parsnip), a small population could establish. In carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot seeds or tissue cultures. However, the bacterium is not likely to persist in the environment in association with commercial annual crops, which are harvested for human consumption prior to setting seed.
- In the absence of a suitable vector, only transient '*Ca. L. solanacearum*' populations are likely to arise. Long-term establishment would depend on the presence of a vector colonising hosts of '*Ca. L. solanacearum*', either already present or introduced at the same time.
- '*Ca. L. solanacearum*' is systemic in the host plant and is seed-borne in carrot (Bertolini et al. 2015). It can only multiply and move in the phloem of the host plant. For '*Ca. L. solanacearum*' to transfer to a suitable host plant, a vector would have to be present in the PRA, acquire the bacterium by feeding on infested plants resulting from infected seeds or tissue cultures and transmit the bacterium to new host plant. No vectors other than *B. trigonica* (Teresani et al. 2015) and *T. apicalis* (Munyaneza et al. 2010b) are currently known on apiaceous crops, and these species do not occur in Australia. It is uncertain whether other psyllids in Australia could act as vectors for '*Ca. L. solanacearum*' in apiaceous crops.
- '*Ca. L. solanacearum*' is likely to require psyllid insect vectors in order to be transmitted to new host plants. This pathogen is transmitted to new host plants by *B. trigonica* and *T. apicalis* (Munyaneza et al. 2010b; Teresani et al. 2015). '*Ca. L. solanacearum*' is vectored by

*Trioza apicalis* in Scandinavian area and parts of Europe, whereas *Bactericera trigonica* vectors this bacterium in Spain. The bacterium has been detected in *B. tremblayi* and *B. nigricornis* collected from carrot and celery fields in Spain (Teresani et al. 2015). However, these vectors are not present in Australia.

- There are no records of any *Bactericera* species in mainland Australia except in Norfolk Island<sup>1</sup> (NIQS 2014). *Trioza apicalis* is an efficient vector of '*Ca. L. solanacearum*' (Alfaro-Fernández et al. 2012b). There are several species of *Trioza* in Australia (DEWHA 2009), however, there are no published records of Australian *Trioza* species feeding on apiaceous crops.
  - Australian *Trioza* species feed on non-apiaceous hosts including Myrtaceae, Euphorbiaceae and Asteraceae (DEWHA 2009). *Trioza banksiae*, *Trioza barrettae*, *Trioza euginae*, *Trioza kentae*, *T. mallotica*, *Trioza oleariae*, *T. pallida* *Trioza percyae*, *Trioza tricornuta* and *T. tristaniae* have pit-gall creating nymphs that feed on plant sap. However, this damage is not similar to the symptoms caused by '*Ca. L.*' species. *Trioza oleari* also feeds on plant sap; however, it is different to the other species of psyllid in that it has free-living nymphs.
- Species of psyllids (other than *B. trigonica* and *T. apicalis*) collected from carrot, celery and potato crops in areas of Spain where '*Ca. L. solanacearum*' is present include *Trioza urticae*, *Ctenarytaina* species, *Cacopsylla* species and *Psylla* species (Teresani et al. 2015). '*Ca. L. solanacearum*' has not been detected in these psyllid species (Teresani et al. 2015).
- In the absence of a vector, the bacterium is unlikely to move from infected plants (resulting from imported seeds or tissue cultures) to new host plants. The bacterium is likely to survive as long as its host plant is present. In case of annual crops, it is likely to depend on a vector to transfer it to alternative hosts to survive the cropless period.

#### **Suitability of the environment**

- The origin of '*Ca. L. solanacearum*' associated with apiaceous crops is not known and is difficult to determine; however, this bacterium has now established in Austria, Germany, France, Morocco, Scandinavian countries and Spain (Haapalainen 2014; Munyaneza et al. 2015). 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. solanacearum*'.
- The extensive occurrence of '*Ca. L. solanacearum*' in various Scandinavian and Mediterranean countries (Haapalainen 2014; Munyaneza et al. 2015) indicates that climatic conditions would be suitable for establishment in Australia. However, without a vector '*Ca. L. solanacearum*' is unlikely to persist in the environment because the pathogen would be unlikely to be transmitted to new hosts.
- '*Ca. L. solanacearum*' is heat sensitive (Munyaneza 2012); therefore, its establishment is likely to be restricted to the temperate southern regions of Australia and it is less likely to establish in the tropical northern regions of Australia.

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<sup>1</sup> *Bactericera cockerelli* has recently been confirmed in Norfolk Island which is isolated from the mainland of Australia by a full quarantine barrier and 1400 km of sea.

- '*Ca. L. solanacearum*' in apiaceous crops was first identified in 2010, but had presumably been associated with some diseases of carrot since at least the 1990s (Font et al. 1999). '*Candidatus Liberibacter solanacearum*' has since been detected in celery and parsnip in Spain and in different psyllid vectors (Cambra et al. 2015; Teresani et al. 2015). The bacterium may be present in more host species because psyllid vectors feed on several plants.
- Five haplotypes of '*Ca. L. solanacearum*' have been identified to date (Nelson et al. 2012; Teresani et al. 2014b). The first two haplotypes associated with solanaceous crops are distributed in Central America, North America and New Zealand (Munyaneza et al. 2007; Secor et al. 2009), while the other haplotypes are associated with carrot and celery crops in Europe (Munyaneza 2010; Alfaro-Fernandez et al. 2012a, b) and Morocco (Tahzima et al. 2014).

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

- '*Ca. L.*' species are obligate parasites of plants and psyllids (Haapalainen 2014).
- '*Ca. L. solanacearum*' multiplies and survives in the phloem of infected host plants and in its psyllid vectors, including *B. trigonica* (Teresani et al. 2015) and *T. apicalis* (Munyaneza et al. 2010b). '*Candidatus Liberibacter solanacearum*' is reliant on infected host plants for survival and is likely to survive as long as the infected plant material survives.
- The survival and multiplication of this bacterium within its host is influenced by temperature. '*Ca. L. solanacearum*' is heat sensitive, but has established across a wide range of climates (Munyaneza 2012).
- Outside of a host plant, '*Ca. L. solanacearum*' is able to survive in the insect vector for a considerable period of time. The life cycle of *T. apicalis* from egg to adult is up to 54 days, depending upon the food supply and ambient temperature (Láska 1974). In addition, *T. apicalis* adults are known to survive seven months when they overwinter on suitable hosts (Valterová et al. 1997).
- On carrot plants, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot seeds and tissue cultures. In the absence of a suitable vector, it is likely that these cases will not lead to long term establishment, but they could lead to transient '*Ca. L. solanacearum*' populations.

#### **4.1.3 Likelihood of spread**

The likelihood that '*Ca. L. solanacearum*', having entered on propagative material (seeds and tissue cultures) and established, will spread in Australia, based on a comparison of factors, in the source and destination areas, considered pertinent to the expansion of the geographic distribution of the pest is **HIGH** (if suitable psyllid vectors are present) or **VERY LOW** (in the absence of suitable psyllid vectors).

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

- The origin of '*Ca. L. solanacearum*' associated with apiaceous crops is not known. However, detection of this bacterium in geographically distant areas with different vectors, triggers questions on the real distribution and pathways for spread of the bacterium. The origin of '*Ca. L. solanacearum*' in Finland is uncertain but symptoms similar to those caused by '*Ca. L.*

*solanacearum*' have previously been reported in Finland (Markkula et al. 1976; Nehlin et al. 1994). Similarly, outbreaks of yellows disease have previously been observed in different regions of Spain, including the Canary Islands (Font et al. 1999), prior to the detection of the bacterium in carrot. Carrot fields in the Canary Islands were heavily infested with the psyllid *B. trigonica* at the same time (Font et al. 1999).

- '*Ca. L. solanacearum*' was first discovered in a carrot crop in Finland (Munyaneza et al. 2010a) and since then has been detected in Norway (Munyaneza 2012a), Sweden (Munyaneza et al. 2012b), Spain (Alfaro-Fernández et al. 2012a), France (Loiseau et al. 2014), Morocco (Tahzima et al. 2014), Austria (EPPO Reporting Service 2015) and Germany (Munyaneza et al. 2015), indicating that the pathogen is able to spread naturally in seed trade over these long distance. The current distribution suggests that the bacterium may be more widely distributed than previously reported (Loiseau et al. 2014). However, it is not present everywhere the vector is present (Teulon et al. 2009). There are similarities in the natural and urban environments of these areas with those in Australia, which suggests that '*Ca. L. solanacearum*' could spread in Australia.
- '*Ca. L. solanacearum*' is known to be heat-sensitive and is inactivated by temperatures above 32 °C (Munyaneza 2012). This indicates that '*Ca. L. solanacearum*' is vulnerable to hot dry conditions (Munyaneza 2012) and would be likely to be restricted in range to the cooler regions of Australia.
- Studies indicate that temperatures at or below 17 °C may significantly slow, but not prevent, '*Ca. L. solanacearum*' development in potato (Munyaneza et al. 2012a). The optimum development of '*Ca. L. solanacearum*' and Zebra chip symptoms in potato plants was observed at a daily temperature regime of 27 °C to 32 °C. At the higher daily temperature regimes of 32 °C to 35 °C and 35 °C to 40 °C, no '*Ca. L. solanacearum*' was detected in potato plants inoculated with the bacterium using insects and no Zebra chip symptom development occurred (Munyaneza et al. 2012a).
- Natural spread of '*Ca. L. solanacearum*' is dependent on the transmission of the bacterium by seeds (Bertolini et al. 2015) and its psyllid vectors (Bertolini et al. 2015). In the absence of psyllid vectors, natural spread of '*Ca. L.*' species is likely to be limited or absent. Natural spread may be achieved though seeds, as this bacterium is seed-borne and seed transmissible in carrot (Bertolini et al. 2015).
- On carrot seeds, the infection may be maintained as long as new carrot seeds are being produced from the line originating from the infected imported carrot seeds. It is likely that these cases will not lead to long distance spread, but rather transient and localised '*Ca. L. solanacearum*' populations only.
- '*Ca. L. solanacearum*' thrives in two very different environments; plant phloem and insect vectors (Nachappa et al. 2014). Therefore, the natural or managed environment is suitable for the natural spread of this bacterium.
- '*Ca. L. solanacearum*' has been detected in three species of *Bactericera* (*B. nigricornis*, *B. tremblayi* and *B. trigonica*) and *T. apicalis*. However, only *B. trigonica* and *T. apicalis* are known to transmit '*Ca. L. solanacearum*' naturally (Haapalainen 2014). This vector specificity suggests it is unlikely that Australian native psyllids would be able to transmit '*Ca. L. solanacearum*'. In the absence of psyllid vectors, long distance spread of '*Ca. L.*' species would rely on human-mediated spread of infected propagative material.

### **Presence of natural barriers**

- From its initial detection in Finland (Munyanzeza et al. 2010a), the bacterium has moved over long distances to Austria, France, Germany, Morocco, Norway, Spain and Sweden (Haapalainen 2014; Munyanzeza et al. 2015).
- Hosts of '*Ca. L. solanacearum*' are present in many parts of Australia. Natural barriers, such as arid areas, mountain ranges, climatic differentials and the potentially long distances between suitable hosts may prevent the long-distance natural spread of this bacterium.
- Carrot, celery and parsnip crops are grown across Australia in isolated regions with long distances often separating commercial production fields. This will aid in the containment and eradication of the vector and/or pathogen if establishment occurs in an isolated growing region.
- '*Ca. L. solanacearum*' is heat sensitive (Munyanzeza et al. 2012a) and arid regions surrounding many production areas in Australia may prove to be natural barriers to the spread of this bacterium. This suggests that the bacterium is also less likely to spread into the warmer arid and tropical regions of Australia.
- A significant natural barrier to spread of '*Ca. L. solanacearum*' is the absence of suitable known insect vectors of the bacterium in Australia. The natural spread of '*Ca. L. solanacearum*' associated with apiaceous crops is achieved by *B. trigonica* (Bertolini et al. 2015) and *T. apicalis* (Munyanzeza et al. 2010b), which are not present in Australia.

### **Potential for movement with commodities**

- Human-mediated movement of plants and plant products is considered the primary mode for the introduction of plant pathogens into new areas. As visual symptoms may not be present, infected propagative material could easily be moved into new areas. The introduction of infected plant material establishes the pathogen in new areas and unregulated movement may accelerate the spread of '*Ca. L. solanacearum*'.
- '*Ca. L. solanacearum*' has the potential to spread from its point of introduction to new areas within Australia by human mediated activities (via trade in propagative material). Carrot, celery and parsnip are grown in various regions of Australia. If imported infected propagative material is distributed throughout production areas, this will help spread '*Ca. L. solanacearum*' throughout Australia.
- The increased trade of propagative material between (and within) countries has led to new opportunities for plant pathogens to spread to new areas (Dehnen-Schmutz et al. 2010; Wingfield et al. 2010; Stenlid et al. 2011). Infection of carrots with '*Ca. L. solanacearum*' in different geographically distant areas, such as Austria, Finland, France, Germany, Morocco, Spain and Sweden (EPPO 2013; Haapalainen 2014; Munyanzeza et al. 2015), suggests that the movement of carrot seeds might be responsible for the introduction of this bacterium into these countries. Therefore, there is potential for the spread of the bacterium through the distribution of infected seeds between carrot growing areas of Australia.
- Plants infected with '*Ca. L. solanacearum*' may be symptomless and could also contribute to the introduction of the bacterium into new areas through nursery stock (Teresani et al. 2014b).

- The bacterium could be spread with the movement and trade of infected host plant material, including seeds and tissue cultures. However, spread of the bacterium by movement of infected planting material will be likely to result in an isolated infection if a vector is not present in the areas where the material is moved.
- In the absence of statutory control, it is likely that '*Ca. L. solanacearum*' will spread within Australia through the trade of seeds (carrot) and tissue cultures (carrot, celery and parsnip) for propagation. Planting of infected seeds and tissue cultures in production fields is likely to introduce '*Ca. L. solanacearum*' into the environment. Climatic conditions in propagation houses and the environment is likely to be sufficient for its survival and spread to new hosts.

#### **Potential natural enemies**

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

#### **Spread potential if known vectors establish in Australia**

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

- Vector-transmitted pathogens rely on complex interactions between the host, the vector and the pathogen for their spread. In plant pathosystems, the spread of a pathogen is highly dependent on the movement and mobility of the vector (Martini et al. 2015).
- '*Ca. L. solanacearum*' associated with apiaceous crops is vectored by *B. trigonica* and *T. apicalis* (Munyaneza et al. 2010a, b; Alfaro-Fernández et al. 2012b). '*Ca. L. solanacearum*' has also been detected in *B. nigricornis* and *B. tremblayi* collected from carrot and celery fields where the bacterium is present (Teresani et al. 2015). These findings on the association of '*Ca. L. solanacearum*' with other psyllids suggest that this bacterium is likely to have more insect vectors than currently known. However, it is most likely that potential vectors are in the closely related *Bactericera* and *Trioza* genera.
- Host selection by insects is based on various stimuli released by the host plant, and consists of a sequence of behavioural responses (Visser 1986; Stadler 1992). Different types of compounds released from carrots have been identified (Buttery et al. 1968; Simon et al. 1980). Host selection in *T. apicalis* seems to be connected to the high release of sabinene and apinene by host plants (Nehlin et al. 1996)
- '*Ca. L. solanacearum*' may initially be introduced to new areas by carrot seeds (Bertolini et al. 2015) and may afterwards be transmitted by different psyllid species in a persistent manner (Teresani et al. 2015).
- Psyllids acquire the bacterium while feeding on infected plants (Haapalainen et al. 2014). Once the psyllid has acquired the bacterium from infected hosts, the vector is likely to maintain the ability to spread the bacterium in a persistent manner (Teresani et al. 2015).
- The presence of psyllid vectors, including *B. trigonica*, can increase the presence of '*Ca. L. solanacearum*' in carrot fields from two percent to close to 100 percent after six months of cultivation (Bertolini et al. 2015).

- '*Ca. L. solanacearum*' associated with apiaceous crops and its vectors (*B. trigonica* and *T. apicalis*) are heat-sensitive (Munyaneza 2012) and distributed in the Mediterranean region, and Northern and Central Europe (Haapalainen et al. 2014).
- Both '*Ca. L. solanacearum*' and its vectors are less likely 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 southern regions and not to the subtropical and tropical northern regions, or arid central regions, of Australia.
- Psyllid vectors feeding on infected host plants are likely to acquire and maintain the bacterium (Haapalainen et al. 2014). The natural spread of '*Ca. L. solanacearum*' by its psyllid vectors will depend on the acquisition period, the latency period of the pathogen in the psyllid prior to transmission and the transmission efficiency.
  - Psyllids acquire the bacterium within two hours of colonising the plant (Munyaneza 2010). The bacterium multiplies in the psyllid and transmission occurs within three days (Nissinen et al. 2014). The symptoms of leaf discolouration in carrot become visible within 1–1.5 months (Nissinen et al. 2007; 2012).
  - '*Ca. L. solanacearum*' titre in the exposed carrot crops correlates with the '*Ca. L. solanacearum*' titre in the psyllids. A high '*Ca. L. solanacearum*' titre in the plants significantly reduced the root weight, while not affecting the number of curled leaves. This suggests that the leaf-curling symptom is caused by the psyllid feeding alone, and that the subsequent leaf discolouration symptom is caused by the multiplication of the bacteria (Nissinen et al. 2014).
- *Trioza apicalis* has a strong preference for its summer host and chooses carrots over other host plants (Rygg 1977; Nehlin et al. 1996). *Trioza apicalis* has one generation per year and overwinters as an adult on conifers, favouring Norway spruce (*Picea abies*) as the winter host (Kristoffersen & Anderbrant 2007). Overwintering adult *T. apicalis* usually start migrating to carrot fields during early summer. They feed and lay eggs on carrot plants during summer (Kristoffersen & Anderbrant 2007). Both the summer and winter hosts of this psyllid are present in Australia.
- The factors triggering the carrot psyllid migration from overwintering hosts to carrots are not known. Terpenes produced in spruce needles may play a role in migration (Schönwitz et al. 1990). Out of the terpenes, limonene was the most effective repellent of psyllids in carrot fields (Nehlin et al. 1996). Therefore, it has been suggested that that changes in the concentration of secondary metabolites in the winter host plant (i.e. Norway spruce) could affect the migration of the carrot psyllid (Nissinen et al. 2008).
- The total number of eggs laid by a mated female psyllid is between one hundred and one thousand (Hodkinson 2009); thus, psyllid populations can grow very rapidly. *Trioza apicalis* females are able to lay up to 900 eggs during their lifespan (Láska 1964 cited in Nissinen et al. 2014) and they need a high amount of nutrients to produce such a high number of eggs. This is likely to increase the time females spend feeding and increase the time during which the females can transmit the '*Ca. L. solanacearum*' bacteria into the plant phloem.
- Psyllids are highly effective dispersers over both short and long distances, although in almost all cases dispersal is wind assisted. The maximum distance that the carrot psyllid can fly is not known (Kristoffersen & Anderbrant 2007). However, *Trioza apicalis* is known to move up to one kilometre to shelter plants to overwinter (Kristoffersen & Anderbrant 2007).

- The '*B. nigricornis* group' (Hodkinson 1981) has polyphagous habits and overlapping areas of distribution and is widely distributed in the Mediterranean region (Ouvrard 2015). The '*B. nigricornis* group' feeds on a variety of herbaceous plants, including beet, cabbage, carrot, onion, parsley, potato (Burckhardt & Lauterer 1997) and celery (Alfaro-Fernández et al. 2012a; Bertolini et al. 2015).
- The '*B. nigricornis* group' is composed of multivoltine species, having two or three generations per year, which feed on carrots and other herbaceous crops (Hodkinson 2009). Eggs are laid on the host foliage and the entire duration of the lifecycle is four to five weeks; but this varies considerably depending on the hosts and temperatures. The adults overwinter in evergreen shrubs (Hodkinson 2009). Short development times and high rates of oviposition allow populations to increase explosively under optimal conditions (Liu & Trumble 2004).
- *Bactericera tremblayi* is associated with Mediterranean climates; however, *B. nigricornis* and *B. trigonica*, which are also found in these regions, are associated with more temperate climates (Teresani et al. 2015). Suitable climates are present in parts of Australia, which is likely to assist in the spread of these vectors.
- The spread of the bacterium in the presence of the vector can be dramatic. For example, in the presence of *B. trigonica*, '*Ca. L. solanacearum*' spread across an entire carrot field to infect 100 percent of the crop (Bertolini et al. 2015). Therefore, the presence of the vector, the bacterium can spread locally very quickly.
- 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 *Bactericera* species and *T. apicalis* to spread '*Ca. L. solanacearum*' to new areas within Australia. However, passive movement of the psyllid, for instance by wind, indicates that infected psyllids could overcome the natural barriers in Australia and spread the bacterium in suitable environments.
- *Bactericera* species feed and reproduce on a wide variety of hosts including carrot, celery and potato, whereas *T. apicalis* preferably feed and breed on carrot, coriander and caraway (Valterová et al. 1997). These hosts are widespread in commercial, natural and urban environments of Australia. This makes it increasingly likely that the vectors would spread '*Ca. L. solanacearum*' to suitable hosts, if introduced to Australia.
- Temperature and humidity may affect both the absolute distribution and the relative breeding success of a psyllid species across its range (Hodkinson 2009). Overlapping host ranges would provide ample opportunity for *B. nigricornis* (which can feed on carrot and celery) to acquire '*Ca. L. solanacearum*' and spread it to healthy hosts.

#### **Spread potential in the absence of known vector**

- In the absence of known vectors, the most likely means of spread of '*Ca. L. solanacearum*' would be through the movement of infected planting material (seeds and tissue cultures). If infected planting material is used it will help spread the bacterium to non-infested areas within Australia.
- It is unlikely that any of the endemic species of psyllid in Australia would be able to vector the bacterium. '*Ca. L. solanacearum*' associated with apiaceous crops as it is only known to be



vectored by the '*B. nigricornis* group' (*B. tremblayi*, *B. nigricornis* and *B. trigonica*) and *T. apicalis* (Alfaro-Fernández et al. 2012b; Nelson et al. 2012; Teresani et al. 2014a).

- If '*Ca. L. solanacearum*' was to establish in Australia, in the absence of vectors, it would be unlikely to lead to the establishment of this bacterium at multiple locations. However, the knowledge of insect populations visiting carrot or celery crops from infected propagative material is required for the identification of putative vector species in Australia. In Spain, '*Ca. L. solanacearum*' has been detected in *B. tremblayi* and *B. nigricornis*.
- Species of psyllid other than the '*B. nigricornis* group' (*B. trigonica*, *B. tremblayi* and *B. nigricornis*) and *T. apicalis*, such as *Trioza urticae*, *Ctenarytaina* species, *Cacopsylla* species and *Psylla* species have been reported on carrot and celery crops in Spain where '*Ca. L. solanacearum*' is known to occur (Teresani et al. 2015). However, '*Ca. L. solanacearum*' was not detected in these psyllid species (Teresani et al. 2015).
  - One unnamed species of *Cacopsylla* and six species of *Ctenarytaina* have been detected in Australia, feeding on species other than apiaceous crops (DEWHA 2009). Additionally, several species of *Trioza* including *Trioza banksiae*, *Trioza barrettae*, *Trioza euginae*, *Trioza kentae*, *T. mallotica*, *Trioza oleariae*, *T. pallida*, *Trioza percyae*, *Trioza tricornuta* and *T. tristaniae* have been recorded on non-apiaceous hosts. Therefore, these psyllids are unlikely to transmit this bacterium to apiaceous crops in Australia.

#### 4.1.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, establishment and spread using the matrix of 'rules' for combining likelihoods shown in Table 2.

- The overall likelihood that '*Ca. L. solanacearum*' will enter Australia on seed (carrot) and tissue cultures (carrot, celery and parsnip), be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia is set out in Table 10.

**Table 10 Overall likelihood of entry, establishment and spread of 'Candidatus Liberibacter solanacearum' on different pathways**

Pathway	Likelihood of			Overall likelihood of entry, establishment and spread
	Entry	Establishment	Spread	
Seeds for sowing	Low	Moderate	High (very low)*	Low (very low)
Tissue cultures	Low	Moderate	High (very low)*	Low (very low)

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

#### 4.1.5 Consequences

The potential consequences of the introduction and spread of '*Ca. L. solanacearum*' associated with apiaceous crops in Australia have been estimated according to the methods described in Table 4. In assessing the potential impact of '*Ca. L. solanacearum*' in Australia, the economic losses caused by this pathogen in Europe were considered.

Reasoning for these ratings is provided below:

Criterion	Estimate and rationale
<b>Direct</b>	
Plant life or health	<p>F—Major significance at the regional level</p> <p>'<i>Ca. L. solanacearum</i>' associated with apiaceous crops (carrot, celery and parsnip) have a significant effect on plant health, life and yield. This bacterium causes yellows decline and vegetative disorders in carrots (Munyaneza et al. 2010a, b) and vegetative disorders in celery (Teresani et al. 2014b). The bacterium/vector complex has caused serious damage to the carrot and celery industry in Europe (EPPO 2013). In Europe, damage to carrots by '<i>Candidatus Liberibacter</i>'-infected carrot psyllids can cause up to 100 percent crop loss (EPPO 2013) without management.</p> <ul style="list-style-type: none"> <li>• Symptoms caused by '<i>Ca. L. solanacearum</i>' in carrot plants include leaf yellowing, bronze or red leaf discolouration, reduced size of the main root and lateral root proliferation (Munyaneza et al. 2011). Other symptoms include stunting, proliferation of dwarfed shoots with bushy tops and a dense hairy growth of secondary roots (Loiseau et al. 2014).</li> <li>• Symptoms caused by '<i>Ca. L. solanacearum</i>' in celery include an abnormal amount of shoots, curling of stems and yellowing (Teresani et al. 2014b).</li> <li>• The economic impact on plant life or health may depend on the extent of symptom expression on carrot and celery. In addition to the yield, the quality of the crop is also affected, with a reduced amount of sugars and increased amounts of phenolic compounds in the root resulting in a bitter taste (Nissinen et al. 2012; Seljåsen et al. 2013).</li> <li>• Vegetative disorders associated with '<i>Ca. L. solanacearum</i>' in carrot producing areas in Spain have caused economic losses in carrot production for the fresh market (Bertolini et al. 2015).</li> </ul>
Other aspects of the environment	<p>B—Minor significance at the local level</p> <p>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.</p> <ul style="list-style-type: none"> <li>• 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. <ul style="list-style-type: none"> <li>– '<i>Ca. L. solanacearum</i>' haplotypes associated with apiaceous crops are unlikely to affect the environment in these ways, as the bacterium is only reported to infect and multiply in carrot, celery, parsnip and their psyllid vectors. The psyllid vectors of this bacterium are not known to occur in Australia.</li> </ul> </li> </ul>
<b>Indirect</b>	
Eradication, control, etc.	<p>E—Significant at the regional level</p> <p>The combination of human induced introductions (Bertolini et al. 2015) and potential human-mediated spread (trade in infected seeds and tissue cultures) makes this a difficult pathogen to eradicate. All known '<i>Candidatus Liberibacter</i>' species are not known to occur in Australia and consequently post-border measures are not currently in place. Outside of Australia, there are few effective control strategies for plant protection against natural '<i>Ca. Liberibacter</i>' species infection.</p> <ul style="list-style-type: none"> <li>• There are no agri-chemicals available for control of '<i>Ca. L. solanacearum</i>' in host crops.</li> <li>• Control of the psyllid vector is the key to limiting the spread impact of pathogen.</li> <li>• The use of '<i>Ca. L. solanacearum</i>'-free carrot seeds for sowing complemented with psyllid control to prevent transmission of the bacterium may be feasible to control the</li> </ul>

Criterion	Estimate and rationale
	<p>bacterium.</p> <ul style="list-style-type: none"> <li>The reduction of the psyllid population is critical, as even vectors with poor transmission efficiency may play a role in spreading the bacterium. For example, high abundance of the vector may compensate for poor transmission efficiency. Insecticidal sprays will be required to manage psyllid populations (Láska 1974; Fischer &amp; Terettaz 2002), which will increase production costs.</li> <li>Growth of host crops in insect-proof facilities could potentially protect crops from this bacterium.</li> </ul>
Domestic trade	<p>D—Significant at the district level</p> <ul style="list-style-type: none"> <li>The presence of '<i>Ca. L. solanacearum</i>' is likely to result in domestic movement restrictions for carrot, celery and parsnip propagative material. Interstate restrictions on seeds and nursery stock may lead to a loss of markets, which would be likely to require industry adjustment.</li> <li>Stringent controls on domestic trade would be required if '<i>Ca. L. solanacearum</i>' became established in Australia. Restrictions might apply to domestic trade in seeds and nursery stock.</li> </ul>
International trade	<p>D—Significant at the district level</p> <ul style="list-style-type: none"> <li>If '<i>Ca. L. solanacearum</i>' became established in Australia, restrictions on Australian exports of seeds (carrot) and nursery stock (carrot, celery and parsnip) would be anticipated. Several countries have established quarantine policies and protocols against plant materials from areas known to have '<i>Ca. L. solanacearum</i>'. Establishment of '<i>Ca. L. solanacearum</i>' in Australia may therefore reduce access to international markets and result in additional phytosanitary requirements that will impose a cost burden.</li> </ul>
Environmental and non-commercial	<p>C—Significant at the local level.</p> <ul style="list-style-type: none"> <li>No direct control measures are available for the bacterium; however, broad-scale chemical treatments directed against known insect vectors may have some impacts on native insects.</li> <li>In Europe, insecticidal sprays are required to manage psyllid populations, as without sprays, substantial losses have been reported (Láska 1974; Tiilikkala et al. 1995; Fischer &amp; Terettaz 2002). Insecticidal sprays used to control psyllid populations may also have some impacts on the environment, including native insects.</li> </ul>

Based on the decision rules described in Table 5, that is, where the potential consequences of a pest with respect to a single criteria have an impact of 'F', the overall consequences are estimated to be **HIGH**.

#### 4.1.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihoods of entry, establishment and spread with the outcome of overall consequences. Likelihood and consequences are combined using the risk estimation matrix shown in Table 11.

**Table 11 Unrestricted risk estimates of '*Candidatus Liberibacter solanacearum*' for different pathways**

Overall likelihood of entry, establishment and spread	Low (very low)
Consequences	High
Unrestricted risk	Moderate (Low)

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

The unrestricted risk estimate for '*Ca. L. solanacearum*' has been assessed as 'moderate' (if vectors are present) and 'low' (if vectors are not present) for seeds (carrot) and tissue cultures (carrot, celery and parsnip), which is above Australia's ALOP. Therefore, risk management measures against '*Ca. L. solanacearum*' are justified for seeds (carrot) and tissue cultures (carrot, celery and parsnip) to reduce risk to meet Australia's ALOP.

## 5 Pest risk management

The IPPC and the WTO recognise the phytosanitary concerns associated with expanding world trade in plant propagative material, including seed. Consequently, international standards have been developed for the safe movement of plants and plant products. The aim of these standards is to reduce the likelihood of the accidental introduction of pests associated with propagative material into new areas through the application of phytosanitary measures. Measures may be applied only where necessary to prevent the introduction and/or spread of quarantine pests. Phytosanitary measures should be applied in a transparent and non-discriminatory manner, and phytosanitary restrictions used only where technically justified and not in lieu of barriers to protect an industry from competition.

The ultimate goal of the phytosanitary measures proposed in this review is to protect Australian agriculture, the economy and environment from the introduction of '*Ca. L. solanacearum*' associated with seeds (carrot) and tissue cultures (carrot, celery and parsnip). To effectively prevent the introduction of '*Ca. L. solanacearum*' associated with apiaceous propagative material, a series of important safeguards, conditions or phytosanitary measures must be in place.

Australia has had emergency measures in place to protect the Australian carrot and celery industries from '*Ca. L. solanacearum*' since October 2014. This PRA reviews the appropriateness of the existing emergency measures, in accordance with ISPM 1: *Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade* (FAO 2011a).

### 5.1 Introduction of emergency measures

The introduction of emergency measures by Australia is consistent with ISPM 1 (FAO 2011a) and IPPC article VII.6 (FAO 2011b). ISPM 1 states that 'contracting parties may adopt and/or implement emergency actions, including emergency measures, when a new or unexpected phytosanitary risk is identified. Emergency measures should be temporary in their application. The continuance of the measures should be evaluated by pest risk analysis or other comparable examination as soon as possible, to ensure that the continuance of the measure is technically justified'. ISPM 5 defines an emergency measure as a phytosanitary measure established as a matter of urgency in a new or unexpected phytosanitary situation (FAO 2015). An emergency measure may or may not be a provisional measure.

The detection of '*Ca. L. solanacearum*' in apiaceous crops (carrot and celery) overseas, systemic infection of hosts and demonstration of the seed-borne nature and seed transmission of '*Ca. L. solanacearum*' in carrot (Bertolini et al. 2014; 2015) created a new phytosanitary situation. Therefore, seed (carrot) and tissue culture (carrot and celery) represented potential pathways for the introduction of this bacterium into Australia. Consequently, Australia introduced emergency measures in October 2014 for imports of carrot seed and carrot and celery tissue cultures to protect the Australian carrot and celery industries.

### 5.1.1 Emergency measures for carrot seeds for sowing (commercial lots)

The introduced emergency measures require that, in addition to general seed for sowing requirements, carrot seed for sowing from all sources should be subject to following conditions:

- Mandatory off-shore or on-shore testing for freedom from '*Ca. L. solanacearum*' using a Polymerase Chain Reaction (PCR) test on a sample of 20,000 seeds; **OR**
- Mandatory off-shore or onshore hot water treatment (50 °C for 20 minutes).

### 5.1.2 Emergency measures for carrot seeds for sowing (small lots)

The introduced emergency measures require that, in addition to general seeds for sowing requirements, small seed lots of carrot seeds for sowing (250 grams or less) from all sources should be subject to following conditions:

- Mandatory on-shore testing for freedom from '*Ca. L. solanacearum*' using a Polymerase Chain Reaction (PCR) test using 20 percent of the seed lot weight; **OR**
- Mandatory off-shore or onshore hot water treatment (50 °C for 20 minutes).

### 5.1.3 Emergency measures for carrot and celery tissue cultures

The introduced emergency measures require that, in addition to general tissue culture requirements, tissue cultures of carrot and celery from all sources should be subject to following conditions:

- Mandatory Phytosanitary Certificate accompanying each consignment endorsed with the additional declaration that the consignment was tested using PCR and found to be free from '*Ca. L. solanacearum*'; **AND**
- Mandatory growth in a closed government post-entry quarantine (PEQ) facility for a minimum of three months for disease screening; **AND**
- Mandatory PCR testing for freedom from '*Ca. L. solanacearum*' during PEQ.

## 5.2 Evaluation of emergency measures

Under the IPPC and WTO SPS Agreement, phytosanitary measures against the introduction of new pests must be technically justified. ISPM 1 states that countries may take appropriate emergency action on a pest posing a potential threat to its territories; however, continuance of the measures should be evaluated by pest risk analysis or other comparable examination as soon as possible, to ensure that the continuance of the measure is technically justified (FAO 2011a). As part of this PRA, the department evaluated the appropriateness of the emergency measures to determine if alternative or additional measures are required for seed (carrot) and tissue cultures (carrot and celery).

### 5.2.1 Seeds for sowing from all sources—carrot (commercial lots)

- **Testing or treatment**—The requirement for mandatory testing by PCR using a 20,000 seed sample **or** heat treatment (50 °C for 20 minutes) are appropriate phytosanitary measures for the safe introduction of carrot seed. The ultimate goal of testing or treatment is to manage

the risk of introducing '*Ca. L. solanacearum*' through trade in seed and to achieve Australia's ALOP. Therefore, the existing requirement of mandatory testing or treatment is supported.

- **Certification**—Consistent with ISPMs 7 and 12, Australia requires that if seed testing or heat treatments are performed off-shore, the exporting country should certify that each consignment has been either tested and found free of '*Ca. L. solanacearum*' or subjected to heat treatment. Phytosanitary certification is used to attest that consignments meet the phytosanitary requirements of the importing country and are conducted in accordance with ISPM 12: *Phytosanitary Certificates*. Phytosanitary certification facilitates safe international trade in plants, plant products and other regulated articles by providing internationally agreed documentation and procedures. Therefore, the existing requirement of certification is supported.
- **On-arrival inspection**—The existing requirement of on-arrival inspection for seed for sowing to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material, animal material and any other extraneous contamination of quarantine concern is supported.

### 5.2.2 Seeds for sowing from all sources—carrot (small lots)

- **Testing or treatment**—The requirement for mandatory testing by PCR (using 20 percent of the seed lot weight) or heat treatment (50 °C for 20 minutes) are appropriate phytosanitary measures for the safe introduction of small seed lots (250 grams or less) of carrot seeds. The ultimate goal of testing or treatment is to manage the risk of introducing '*Ca. L. solanacearum*' through trade in seeds and to achieve Australia's ALOP. Therefore, the existing requirement of mandatory testing or treatment is supported.
- **Certification**—Consistent with ISPMs 7 and 12, Australia requires that if heat treatment is performed off-shore, the exporting country should certify that each consignment has been either tested and found free of '*Ca. L. solanacearum*' or subjected to heat treatment. Phytosanitary certification is used to attest that consignments meet the phytosanitary requirements of the importing country and are conducted in accordance with ISPM 12: *Phytosanitary Certificates*. Phytosanitary certification facilitates safe international trade in plants, plant products and other regulated articles by providing internationally agreed documentation and procedures. Therefore, the existing requirement of certification is supported.
- **On-arrival inspection**—The existing requirement of on-arrival inspection for seeds for sowing to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material, animal material and any other extraneous contamination of quarantine concern is supported.

### 5.2.3 Tissue cultures from all sources—carrot and celery

- **Pre-export testing for freedom from '*Ca. L. solanacearum*'**—The requirement for the consignment to be accompanied by a Phytosanitary Certificate with the additional declaration that the consignment has been found to be free from '*Ca. L. solanacearum*' by PCR testing is supported.
- **On-arrival inspection**—The existing requirement of on-arrival inspection for imported tissue cultures to verify freedom from live insects, soil, disease symptoms, prohibited seeds,



other plant material, animal material and any other extraneous contamination of quarantine concern is supported.

### 5.3 Proposed risk mitigation measures

Australia considers that the emergency measures, introduced in October 2014, are adequate to mitigate the risk posed by '*Ca. L. solanacearum*' associated with apiaceous propagative material (seed and tissue cultures). Since the introduction of the emergency measures, parsnip has been reported as a natural host of '*Ca. L. solanacearum*'. Therefore, these emergency measures are proposed to become the standard conditions for the importation of carrot, celery and parsnip propagative material into Australia from those countries that do not regulate '*Ca. L. solanacearum*' associated with apiaceous crops. The department also proposes that consignments of unknown health be permitted importation into Australia, subject to alternative import conditions. The recommended conditions for apiaceous propagative material are summarised below.

#### 5.3.1 Seeds for sowing from all sources—carrot (commercial lots)

- **Testing**—mandatory PCR testing off-shore or on-shore using 20,000 seeds to verify freedom '*Ca. L. solanacearum*'; **OR**
- **Heat treatment**—mandatory off-shore or on-shore heat treatment (50 °C for 20 minutes); **AND**
- **Certification**—seed lots tested off-shore or treated off-shore must be accompanied by an official government Phytosanitary Certificate endorsed with the following additional declaration:
  - 'The carrot seed in the consignment was tested for '*Candidatus Liberibacter solanacearum*' and found to be free of the bacterium using Polymerase Chain Reaction (PCR) test method on a sample of 20,000 seeds'; **OR**
  - 'The carrot seed in the consignment was treated at a minimum temperature of 50 °C for at least 20 minutes'.
- **On-arrival inspection**—commercial seed lots must be subject to on-arrival inspection to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem material, fruit pulp, pod material etc.), animal material (e.g. animal faeces, feathers etc.) and any other extraneous contamination of quarantine concern.

#### 5.3.2 Seeds for sowing from all sources—carrot (small lots)

Seed lots of 250 grams or less are considered a small seed lot.

- **Testing**—mandatory PCR testing off-shore or on-shore using 20 percent of the seed lot weight or 20,000 seeds to verify freedom '*Ca. L. solanacearum*'; **OR**
- **Heat treatment**—mandatory off-shore or on-shore heat treatment (50 °C for 20 minutes); **AND**
- **Certification**—seed lots tested off-shore or treated off-shore must be accompanied by an official government Phytosanitary Certificate endorsed with the following additional declaration:

'The carrot seed in the consignment was tested for '*Candidatus Liberibacter solanacearum*' and found to be free of the bacterium using Polymerase Chain Reaction (PCR) test method on a sample size of 20 percent of the seed lot'; **OR**

'The carrot seed in the consignment was tested for '*Candidatus Liberibacter solanacearum*' and found to be free of the bacterium using Polymerase Chain Reaction (PCR) test method on a sample size of 20,000 seeds' **OR**

'The carrot seed in the consignment was treated at a minimum temperature of 50 °C for at least 20 minutes'.

- **On-arrival inspection**—small seed lots must be subject to on-arrival inspection to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem material, fruit pulp, pod material etc.), animal material (e.g. animal faeces, feathers etc.) and any other extraneous contamination of quarantine concern.

### 5.3.3 Tissue cultures—carrot, celery and parsnip

In addition to proposing the continuance of the current import conditions for tissue cultures of known health, the department proposes an alternative set of conditions for tissue cultures of unknown health. All imported tissue cultures should be well rooted prior to arrival as this helps in their establishment out of agar into the growth media.

#### Tissue cultures (known health)—carrot, celery and parsnip

- **Mandatory testing (off-shore)**—imported tissue cultures must be subject to off-shore PCR testing for freedom from '*Ca. L. solanacearum*' **AND** a Phytosanitary Certificate with the additional declaration that the testing has been conducted in accordance with Australia's requirements.
- **On-arrival inspection**—imported tissue cultures must be subject to on-arrival inspection to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem material, fruit pulp, pod material etc.), animal material (e.g. animal faeces, feathers etc.) and any other extraneous contamination of quarantine concern.

#### Tissue cultures (unknown health)—carrot, celery and parsnip

- **On-arrival inspection**—imported tissue cultures must be subject to on-arrival inspection to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem material, fruit pulp, pod material etc.), animal material (e.g. animal faeces, feathers etc.) and any other extraneous contamination of quarantine concern.
- **Growth in PEQ with disease screening and testing**—imported tissue cultures must be grown in a closed government PEQ facility for a minimum of three months with disease screening and testing using PCR to verify freedom from '*Ca. L. solanacearum*'. PCR testing will assist in the detection of the bacterium in both symptomatic and asymptomatic plants.
  - The department proposes that tissue cultures that have not been certified to be free from this bacterium by PCR testing by the government of the exporting country should be subject to growth in a government closed PEQ facility for a minimum period of three months, with disease screening and testing.

The proposed risk mitigation measures for the importation of carrot, celery and parsnip propagative material are summarised in Table 12.

**Table 12 Proposed risk mitigation measures for the importation of carrot, celery and parsnip propagative material**

<b>Proposed risk mitigation measures</b>	<b>Carrot seeds for sowing</b>	<b>Carrot, celery and parsnip tissue cultures (known health)</b>	<b>Carrot, celery and parsnip tissue cultures (unknown health)</b>
<b>Pre-border</b>			
Off-shore testing	<b>Yes*</b> (off-shore or on-shore PCR test)	<b>Yes</b> (PCR test)	No
Off-shore treatment	<b>Yes*</b> (off-shore or on-shore heat treatment)	No	No
Phytosanitary Certification	<b>Yes*</b> (if testing or treatment conducted off-shore)	<b>Yes</b> (with additional declaration for freedom from 'Ca. L. solanacearum')	No
<b>Border</b>			
On-arrival inspection	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
On-shore PCR testing	<b>Yes*</b> (off-shore or on-shore PCR test)	No	No
On-shore treatment	<b>Yes*</b> (off-shore or on-shore heat treatment)	No	No
<b>Post-border</b>			
Growth in PEQ	No	No	<b>Yes</b>
Disease screening during PEQ	No		<b>Yes</b>
On-shore testing during PEQ	No		<b>Yes</b> (PCR test)

\* Carrot seeds for sowing must be subject to either mandatory heat treatment or mandatory PCR testing. Heat treatment or PCR testing may be conducted off-shore or on-shore. A Phytosanitary Certificate is only required for consignments that have been subject to off-shore PCR testing or off-shore heat treatment.

## 6 Conclusion

The findings of this pest-initiated risk analysis are based on a comprehensive analysis of relevant scientific and other appropriate literature on '*Ca. L. solanacearum*' associated with apiaceous crops. '*Ca. L. solanacearum*' meets the IPPC definitions of a quarantine pest, that is '*potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled*' (FAO 2015). The PRA provides technical justification that this bacterium meets the IPPC definition of a quarantine pest and that the introduction of emergency measures were in accordance with international phytosanitary standards.

The department considers that the emergency measures are adequate to mitigate the risk posed by '*Ca. L. solanacearum*' associated with seed (carrot) and tissue cultures (carrot and celery). More recently, parsnip was reported as a natural host of '*Ca. L. solanacearum*'; consequently, these emergency measures are extended to parsnip tissue cultures.

The emergency measures are proposed to become the standard conditions to import carrot, celery and parsnip propagative material into Australia. The only proposed changes to the emergency measures is to provide the option for small seed lots to be tested off-shore; and alternative conditions for tissue cultures of unknown health status. The proposed import conditions for carrot, celery and parsnip propagative material are summarised below.

**Seeds for sowing (commercial lots) (carrot):** mandatory off-shore or on-shore molecular testing (using 20,000 seeds) **OR** mandatory off-shore or on-shore heat treatment (50 °C for 20 minutes); **AND** a Phytosanitary Certificate with the additional declaration that the mandatory treatment or testing has been conducted in accordance with Australia's requirements.

**Seeds for sowing (small lots [250 grams or less]) (carrot):** mandatory off-shore or on-shore molecular testing (using 20 percent of the seed lot weight or 20,000 seeds) **OR** mandatory off-shore or on-shore heat treatment (50 °C for 20 minutes); **AND** a Phytosanitary Certificate with the additional declaration that the mandatory treatment or testing has been conducted in accordance with Australia's requirements.

**Tissue cultures of known health (carrot, celery and parsnip):** mandatory off-shore molecular testing; **AND** a Phytosanitary Certificate with the additional declaration that the testing has been conducted in accordance with Australia's requirements.

**Tissue cultures of unknown health (carrot, celery and parsnip):** mandatory growth in a closed government post-entry quarantine (PEQ) facility for disease screening; **AND** mandatory PCR testing for freedom from '*Ca. L. solanacearum*'.



## Appendix A: Stakeholders comments on emergency measures

The Department of Agriculture and Water Resources notified international trading partners on 21 August 2014 that emergency measures against '*Ca. L. solanacearum*' associated with carrot and celery propagative material from all countries would be in place from 20 October 2014 (G/SPS/N/AUS/345). The Department of Agriculture and Water Resources received several written responses from stakeholders, including seed companies, international and domestic seed federations and National Plant Protection Organizations (NPPOs).

A summary of issues raised by stakeholders is provided below.

### Issues raised by stakeholders in response to emergency measures

#### Questions in relation to '*Candidatus Liberibacter solanacearum*'

##### 1. Geographical distribution of '*Ca. L. solanacearum*' associated with apiaceous crops

'*Candidatus Liberibacter solanacearum*' associated with apiaceous crops was first reported to occur in Finland (Munyaneza et al. 2010a), Sweden (Munyaneza et al. 2012b) and Norway (Munyaneza et al. 2012c). These were the first reports of '*Ca. L. solanacearum*' being associated with non-solanaceous species and the first reports of this pathogen outside of North America, Central America and New Zealand. Subsequently, '*Ca. L. solanacearum*' was reported on carrot from the Canary Islands and mainland Spain (Alfaro-Fernández et al. 2012a, b). The same pathogen was reported on celery in Spain (EPPO 2012; Teresani et al. 2014b). More recently, '*Ca. L. solanacearum*' was detected in carrots in Morocco (Tahzima et al. 2014), France (Loiseau et al. 2014) Germany (Munyaneza et al. 2015) and Austria (EPPO Reporting Service 2015) and parsnip in Spain (Cambra et al. 2015).

The isolates of '*Ca. L. solanacearum*' from different geographical areas were found to represent different haplotypes (Nelson et al. 2011). Based on molecular studies, five haplotypes of '*Ca. L. solanacearum*' have been characterised (Nelson et al. 2011; 2012). '*Ca. L. solanacearum*' isolates from North and Central America represent haplotypes A and B, which infect solanaceous plants and are transmitted by *B. cockerelli* (Hansen et al. 2008). The isolates from New Zealand belong to the western North American haplotype A. '*Ca. L. solanacearum*' associated with a carrot disease in Northern Europe represent haplotype C (Nelson et al. 2011; 2013), and '*Ca. L. solanacearum*' isolates from carrot and celery plants in Spain and Morocco represent haplotypes D and E (Nelson et al. 2013; Tahzima et al. 2014; Teresani et al. 2014b). It is suspected that '*Ca. L. solanacearum*' associated with apiaceous crops is likely to be more widespread in parts of Northern and Central Europe where psyllid vectors (*Bactericera* species and *Trioza apicalis*) occur.

##### 2. Role of seeds in spreading '*Ca. L. solanacearum*'

The bacterium was first detected in Finland in 2010 (Munyaneza et al. 2010a) and since then has been reported from Austria (EPPO Reporting Service 2015), France (Loiseau et al. 2014), Germany (Munyaneza et al. 2015), Morocco (Tahzima et al. 2014), Norway (Munyaneza 2012), Spain (Alfaro-Fernández et al. 2012a) and Sweden (Munyaneza et al. 2012b). Infection of carrots



with '*Ca. L. solanacearum*' in geographically distant areas and countries in Europe suggests that carrot seeds might be the first source of inoculum in these countries.

Australia acknowledges that psyllids are highly effective dispersers over both short and long distances, although in almost all cases psyllid dispersal is wind assisted. *Trioza apicalis* moves up to one kilometre (Kristoffersen & Anderbrant 2007), indicating the psyllid is able to spread the disease from infected plants to healthy ones. The maximum distance that the carrot psyllid can fly is not known (Kristoffersen & Anderbrant 2007). However, it is unlikely that the bacterium was spread by psyllids carrying the bacterium to all of these countries. Therefore, carrot seeds are likely to be the first source of inoculum in these countries.

### 3. Seed transmission of '*Ca. L. solanacearum*' in carrot

'*Candidatus Liberibacter solanacearum*' associated with carrot is transmitted by seeds (Bertolini et al. 2014). '*Candidatus Liberibacter solanacearum*' was detected in several commercial seed lots produced in France and Spain (Table 13). The detection in various cultivars indicates that the bacterium is widespread and can persist in the seed for a long time, as the bacterium was tested in seed lots produced in different years (Bertolini et al. 2015).

**Table 13** Detection of '*Candidatus Liberibacter solanacearum*' in different seed lots

Seed lot	Production area	Cultivar
06/2013	Spain	Bangor
09/2013	Spain	Bolero
10/2012	France	CAC 3075
11/2012	France	Amsterdam
13/2012	Spain	Carboli
16/2013	Spain	Elegance
20/2013	Spain	Exelso
23/2010	Spain	Maestro
30/2012	Spain	Maestro
34/2013	Spain	Maestro
37/2013	Spain	Musico
43/2013	Spain	Newhall
52/2013	Spain	Soprano
54/2013	Spain	Yaya

Modified from Bertolini et al. 2015

In a study by Bertolini et al. (2015), PCR positive seed lots were grown in insect-proof P2 level containment greenhouses to study seed to seedling transmission. Seed to seedling transmission was demonstrated in three cultivars namely Maestro, Amsterdam and CAC 3075 (Bertolini et al. 2015). '*Candidatus Liberibacter solanacearum*' was consistently detected in all four seed lots tested after 150 days cultivation in the glasshouse (Bertolini et al. 2014). Therefore, '*Ca. L. solanacearum*' is able to be transmitted to the next generation by infected seeds.

### 4. Host range of '*Ca. L. solanacearum*'

'*Ca. L. solanacearum*' is an obligate parasite of plants (carrot, celery and parsnip) and psyllids (*Bactericera* species and *Trioza apicalis*); and is only able to multiply inside its eukaryotic hosts (Haapalainen 2014). '*Candidatus Liberibacter*' species life cycle includes alternating between the

plant and insect hosts. Insect hosts are infected by feeding on the phloem of an infected plant. Once in the insect host, '*Candidatus Liberibacter*' species move from the gut to haemolymph and then to the salivary glands, infected vectors then infect a new plant host during feeding.

### 5. Psyllid vectors and their role in spreading '*Ca. L. solanacearum*'

'*Candidatus Liberibacter solanacearum*' associated with apiaceous crops is transmitted by different psyllid species (Munyaneza et al. 2011; 2012a, b; Alfaro-Fernández et al. 2012a, b; Haapalainen 2014). In Northern and Central Europe, '*Ca. L. solanacearum*' is transmitted by *Triozia apicalis* (Nelson et al. 2011) and in Mediterranean regions it is transmitted by *Bactericera trigonica* (Alfaro-Fernández et al. 2012b; Nelson et al. 2012; Teresani et al. 2014a, b).

'*Candidatus Liberibacter solanacearum*' has also been detected in *B. nigricornis* and *B. tremblayi* collected from carrot and celery fields where the bacterium is present (Teresani et al. 2015); therefore, these psyllids are potential vectors of the bacterium (Teresani et al. 2015). The association of '*Ca. L. solanacearum*' with other psyllids suggests that this bacterium is likely to have more insect vectors than is currently known.

Psyllids acquire the bacterium while feeding on infected plants (Haapalainen et al. 2014) and are infected with the bacterium within two hours of colonising the plant (Munyaneza 2010). The bacterium multiplies in the psyllid and transmission is able to occur within three days (Nissinen et al. 2014). Once the psyllid has acquired the bacterium from infected hosts, the vector is likely to maintain the ability to spread the bacterium in a persistent manner (Teresani et al. 2015).

The '*B. nigricornis* group' is polyphagous and its members have overlapping areas of distribution throughout the Mediterranean region (Hodkinson 1981; Ouvrard 2015). This group feeds on a variety of herbaceous plants, including beet, cabbage, carrot, onion, parsley, potato and celery (Burckhardt & Lauterer 1997; Alfaro-Fernández et al. 2012a; Bertolini et al. 2015). Spread of the bacterium in the presence of the vector can be dramatic. For example, in the presence of the vector (*B. trigonica*), the disease incidence increased from two percent to 100 percent after six months of carrot cultivation (Bertolini et al. 2015).

### 6. Risk of '*Ca. L. solanacearum*' in symptomless plants

'*Candidatus Liberibacter solanacearum*' associated with apiaceous crops has been detected in symptomless plants (Loiseau et al. 2014). '*Candidatus Liberibacter solanacearum*' was detected in seeds produced in France where the bacterium was detected in both symptomatic and asymptomatic plants. Therefore, the risk of '*Ca. L. solanacearum*' in asymptomatic plants may be higher as the symptomless nature of '*Ca. L. solanacearum*' may contribute to the inadvertent propagation and distribution of infected material that may help spread '*Ca. L. solanacearum*' to other countries.

### 7. Koch's postulates for '*Ca. L. solanacearum*'

Koch's postulates to identify the causative agent of a particular disease include:

1. The organism must be regularly associated with the disease;
2. The organism must be isolated from the diseased host and grown in culture;

3. The disease must be reproduced when a pure culture of the organism is introduced into a healthy, susceptible host; and
4. The same organism must be re-isolated from the experimentally infected host.

Despite the importance of Koch's postulates in the identification of causative agents, there are certain limitations. For example, the application of nucleic acid-based methods of microbial identification has made Koch's postulates less applicable. Nucleic acid-based methods, such as PCR, are very sensitive and can detect small numbers of microbes, even if they occur in the absence of disease symptoms.

Australia recognises that traditional Koch's postulates for this pathogen have not been conclusively fulfilled. The prefix '*Candidatus*' is used in a unique trinomial name system, which indicates that the bacterium is unculturable (Jagoueix et al. 1996). Therefore, postulate numbers two, three and four cannot be fulfilled for '*Ca. L.*' species as these bacteria are to-date non-culturable *in vitro* (Munyaneza 2012). However, modified Koch's postulates support the etiological role of '*Ca. L. solanacearum*' associated with apiaceous crops. This bacterium is consistently associated with diseases in carrot and celery (Munyaneza et al. 2011; 2012a, b; Alfaro-Fernández et al. 2012a, b; Haapalainen 2014). Psyllids have been collected from '*Ca. L. solanacearum*'-infected apiaceous crops and celery fields and have been used to infect healthy hosts (Nelson et al. 2011; Teresani et al. 2015). The bacterium has been detected by PCR in plant hosts and psyllid vectors, and the bacterium has been observed in phloem tissue through Transmission and Scanning Electron Microscopes (TEM and SEM). Therefore, Australia considers that modified Koch's postulates have been fulfilled.

#### **8. Demonstration of seed transmission of '*Ca. L. solanacearum*'**

'*Candidatus Liberibacter solanacearum*' has been demonstrated to be seed transmitted in carrots. PCR positive carrot seed lots grown in insect-proof P2 level containment greenhouses resulted in infected seedlings (Bertolini et al. 2015). '*Candidatus Liberibacter solanacearum*' was consistently detected in all four seed lots tested after 150 days cultivation in the glasshouse (Bertolini et al. 2014). Full symptoms have also been shown to occur in carrot plants grown from seeds that test positive to '*Ca. L. solanacearum*' (Bertolini et al. 2015). Therefore, transmission has been demonstrated for carrot seeds. This type of transmission is called 'direct transmission', as the bacterium is carried by the seeds and is transmitted to next generation.

#### **9. Inoculum density of '*Ca. L. solanacearum*' and symptom expression**

'*Candidatus L. solanacearum*' has been shown to cause curling and discolouration of carrot leaves, and overall reduction of plant and root growth. Leaf discolouration and root size reduction symptoms are correlated with colonisation of the carrot phloem vessels by the bacterium. The bacteria systemically colonise the phloem vessels throughout the plant, and proliferate to high density, filling the sieve cells (Haapalainen et al. 2014). However, the carrot plants response to the bacteria is variable. While some plants seem to be able to resist the infection, some of the infected plants show few visible symptoms while others show very clear discolouration symptoms and yield reduction (Haapalainen et al. 2014).

## Questions in relation to risk and impact

### 1. Symptoms caused by the psyllid and '*Ca. L. solanacearum*'

For decades, *T. apicalis* has been a serious pest of carrots in northern and central Europe (Nissinen et al. 2007; Meadow 2010; Munyaneza 2010). Insecticide treatment is an economic necessity in Norway, Sweden, Denmark (where carrot growing was impossible in the 1920s) and Latvia (where about 70 percent of the crop was lost in 1922; Ozols 1925 cited in Láska 2011). In central Europe, Germany has experienced up to 50 percent crop loss and some districts of Czechoslovakia have seen up to 83 percent crop loss (Láska 1974). In Switzerland, the recommendation is that one spray during the season is sufficient (Fischer & Terettaz 2002). Similarly, outbreaks of yellows disease were observed in different regions of Spain, including the Canary Islands (Font et al. 1999). Carrot fields in the Canary Islands were heavily infested with the psyllid *B. trigonica* (Font et al. 1999). Plant symptoms included small leaves with yellowing and reddening discoloration, proliferation of leaves and small roots; and deformation, reduction and early senescence of roots. However, little was known on the mechanisms by which psyllids (*B. trigonica* and *T. apicalis*) cause damage to carrots.

It was widely believed that psyllids affect carrots by injecting toxic saliva into the plants (Markkula & Laurema 1971; Markkula et al. 1976; Nehlin et al. 1994). However, it was recently discovered that psyllids were the vectors of '*Ca. L. solanacearum*' (Munyaneza et al. 2010a, b; Munyaneza et al. 2012a, b). Symptomatic plants and *Bactericera trigonica* collected from carrot fields showing yellows disease from the Canary Islands (Font et al. 1999) were not tested for this liberibacter, as this bacterium was not suspected to be present then (Munyaneza et al. 2010a). However, recent studies have reported '*Ca. L. solanacearum*' in carrot crops and *B. trigonica* in the Canary Islands and other areas in Spain (Alfaro-Fernández et al. 2012a; Teresani et al. 2014b). Symptoms observed in carrots in the Canary Islands (Font et al. 1999) are very similar to those often observed in *T. apicalis*-'*Ca. L.*' affected carrots in Finland (Munyaneza et al. 2010a). These results suggest that plant symptoms observed in the surveyed fields are most likely associated with *T. apicalis* feeding and '*Ca. L. solanacearum*' infection.

### 2. Absence of known vectors in Australia and emergency measures for the risk

Australia acknowledges that the known vectors (*B. trigonica* and *T. apicalis*) of '*Ca. L. solanacearum*' are absent from Australia. '*Candidatus Liberibacter solanacearum*' has also been detected in other psyllids, including *B. nigricornis* and *B. tremblayi*, indicating that this bacterium is likely to have more insect vectors than are currently known. There are several species of psyllids that are native to Australia that may be potential vectors of '*Ca. L. solanacearum*'. Furthermore, there are psyllid species present in Australia that belong to the same genera as the vectors identified overseas.

The emergency measures currently in place are considered appropriate to mitigate the risk of accidental introduction of '*Ca. L. solanacearum*' associated with apiaceous crops into Australia. Australia already has measures in place to prevent the introduction of other bacterial pathogens of quarantine concern, regardless of whether the vectors are present, or not (for example Citrus greening ('*Ca. L.*' species) and Zebra chip ('*Ca. L. solanacearum*')).

## Questions in relation to detection methods

### 1. Detection and differentiation of dead and alive bacterial cells

Australia acknowledges that PCR can detect and quantify the bacterial genome but does not differentiate between alive or dead bacterial cells. However, DNA intercalating dyes such as ethidium monoazide (EMA) or propidium monoazide (PMA) have been used to detect and quantify DNA from only live cells (Nocker & Camper 2006; Trivedi et al. 2009; Nocker et al. 2007; Temple et al. 2013). Bertolini et al. (2015) used PMA to quantify live '*Ca. L. solanacearum*' cells in carrot seeds. This study found that the majority (about 95 percent) of the seeds' bacterial population was dead. However, the live cells (5 percent) were enough to cause infection in carrot seedlings germinated from infected seeds (Bertolini et al. 2015). Similar results have been reported for '*Ca. L. asiaticus*' (83 percent dead cells) after treatment with ethidium monoazide (Trivedi et al. 2009). This also suggests that the detection of dead bacterial cells in a sample can be an indication that low levels of live bacterial cells are present. Therefore, Australia considers that it is not appropriate to differentiate between live and dead cells when testing for the presence of '*Ca. L. solanacearum*' in carrot seeds.

## Questions in relation to emergency measures

### 1. Sample size of 20,000 seeds to verify freedom from '*Ca. L. solanacearum*'

In accordance with ISPM 7, 12 and 23, Australia asked trading partners to inspect carrot seed shipments and to certify that the shipment is free from '*Ca. L. solanacearum*'. Inspection is a practical measure for visible pests or for pests which produce visible symptoms. However, '*Ca. L. solanacearum*' infects the seed without visual symptoms; therefore, visual inspection is insufficient. Consistent with ISPM 11 (FAO 2013), Australia selected pathogen testing of a sample drawn from the consignment to verify freedom from '*Ca. L. solanacearum*' associated with carrot seeds. Seed health testing is an important means of reducing the introduction of pathogens. Direct testing (isolation of the pathogen) or indirect testing (detection of proteins [serological] or detection of nucleic acid [PCR]) methods are used for seed health testing. Australia opted for PCR as a sensitive and less time consuming procedure for the detection of '*Ca. L. solanacearum*' in carrot seeds.

It is expected that some infected seed lots will contain very few infected seeds and consist mostly of healthy seeds. To detect the infected seeds in these lots, substantial seed samples need to be tested. Any infected seeds can introduce the pathogen to new areas. Therefore, tests for seed-borne pathogens are frequently done on samples of 10,000 to 50,000 seeds. For example, testing samples of 30,000 crucifer seeds is the industry standard for detecting *Xanthomonas campestris* pv. *campestris*, and 10,000 to 20,000 carrot seeds is the standard for detecting *Xanthomonas campestris* pv. *carotae* (APHIS 2001; De Boer et al. 2007). Testing samples of 20,000 bean seeds is the standard for detecting *Pseudomonas syringae* pv. *phaseolicola* (Agarwal & Sinclair 1996). A test of 10,000 to 50,000 tomato seeds is routinely used to detect *Clavibacter michiganensis* subsp. *michiganensis* (van Vaerenbergh 2013). A test of 30,000 lettuce seeds is used by other NPPOs to detect *Lettuce mosaic virus* (APHIS 2001). Monsanto tests vegetable seeds for bacterial and viral pathogens using samples of 30,000 or 50,000 seeds (Monsanto 2014). Australian laboratories test tomato and capsicum seeds for viroids using samples of 20,000 seeds.

Australia therefore considers that using a 20,000 seed sample to detect a low level of '*Ca. L. solanacearum*' in carrot seeds is considered justified.

## 2. Heat treatment (50 °C for 20 minutes) for carrot seeds

ISPM 5 defines treatment as an 'official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalisation' (FAO 2015). Consistent with ISPM 5, Australia introduced heat treatment as a measure applied to consignments prior to export or on-arrival. The requirement or application of phytosanitary treatments to regulated articles is a phytosanitary measure used to prevent the introduction and spread of regulated pests. ISPM 28 *Phytosanitary treatments for regulated pests* provides guidelines on the use of treatments to manage pest risk.

Australia acknowledges that there is no specific scientific publication indicating that heat treatment is effective in eliminating '*Ca. L. solanacearum*' from the carrot seeds. However, temperature has a significant effect on the development of '*Ca. L.*' species. Generally, '*Ca. L.*' species are heat sensitive (Aubert 1987; Bové et al. 2008; Lopes et al. 2009), except for '*Ca. L. asiaticus*', which is heat-tolerant (Lopes et al. 2009). Studies indicate that '*Ca. L. solanacearum*' is heat sensitive and the pathogen was not detected in plants grown at temperature regimes of 32 to 35 °C and 35 to 40 °C (Munyaneza et al. 2012a). Based on these studies, Australia introduced heat treatment for carrot seeds.

Australia currently uses hot water treatment at 50 °C for at least 20 minutes to treat '*Ca. L.*' species in citrus seeds. Carrot seeds are generally treated at 50 °C for 20 minutes to control *Alternaria* species and bacterial blight (Floyd & Melvin-Carter 2005). McGrath (2005) also recommends hot water treatment of carrot seeds for 50 °C for 20 minutes to control bacterial pathogens.

Australia therefore considers that using heat treatment (50 °C for 20 minutes) as an alternative to PCR testing of carrot seeds is considered justified.

## 3. Emergency measures to cover carrot seeds from all countries

Outbreaks of yellows disease were observed in different regions of Spain, including the Canary Islands (Font et al. 1999). Carrot fields in the Canary Islands were heavily infested with the psyllid *Bactericera trigonica* (Font et al. 1999). Plant symptoms included small leaves with yellowing and reddening discoloration; proliferation of leaves and small roots; and deformation, reduction and early senescence of roots. Symptoms observed on carrots in the Canary Islands (Font et al. 1999) were very similar to often observed in *T. apicalis*-'*Ca. L.*' affected carrots in Finland (Munyaneza et al. 2010a). Symptomatic plants and *B. trigonica* collected from carrot fields showing yellows disease from the Canary Islands (Font et al. 1999) were not tested for this liberibacter, as this bacterium was not suspected to be present then (Munyaneza et al. 2010a). However, recent studies detected '*Ca. L. solanacearum*' in carrot crops and *B. trigonica* in the Canary Islands and other areas in Spain (Alfaro-Fernández et al. 2012a; Teresani et al. 2014b).

'*Ca. L. solanacearum*' associated with carrot is seed-borne and seed transmissible (Bertolini et al. 2015). The bacterium was first detected in Finland in 2010 (Munyaneza et al. 2010a) and since then has been reported from Austria (EPPO Reporting Service 2015), France (Loiseau et al.

2014), Germany (Munyaneza et al. 2015), Morocco (Tahzima et al. 2014), Norway (Munyaneza 2012), Spain (Alfaro-Fernández et al. 2012a) and Sweden (Munyaneza et al. 2012b). This indicates that the bacterium is likely to have spread to these areas with carrot seeds. The origin and exact distribution of this bacterium is difficult to determine, with an increasing number of countries reporting the detection of this bacterium in carrot and celery crops.

Carrot seeds are regularly traded internationally and production (breeding, testing, multiplication and counter seasonal multiplication) normally occurs at multiple locations. Global trade in seeds and propagative material increases the potential for the introduction of new pathogens. Movement of seeds between countries may result in the unintentional spread of this bacterium, particularly if other countries do not have measures in place to address this bacterium. Therefore, Australia applied emergency measures for carrot seeds from all countries.



## Appendix B: Additional quarantine pest data

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<b>Quarantine pest</b>	'Candidatus Liberibacter solanacearum' Leifiting et al. (haplotypes C, D and E) [Rhizobiales: Rhizobiaceae]
Synonyms	
Common name(s)	Yellows decline and vegetative disorders
Main hosts	<i>Apium graveolens</i> , <i>Daucus carota</i> , <i>Pastinaca sativa</i>
Distribution	Austria, Finland, France, Germany, Morocco, Norway, Spain and Sweden

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## Glossary

<b>Term or abbreviation</b>	<b>Definition</b>
Additional declaration	A statement that is required by an importing country to be entered on a phytosanitary certificate and which provides specific additional information on a consignment in relation to regulated pests (FAO 2015).
Appropriate level of protection (ALOP)	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 2015).
Area of low pest prevalence	An area, whether all of a country, part of a country, or all parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures (FAO 2015).
Arthropod	The largest phylum of animals, including the insects, arachnids and crustaceans.
Casual plant	A plant on which adult psyllids land actively or passively, and on which adults may probe the plant but do not feed.
Consignment	A quantity of plants, plant products 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 2015).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO 2015).
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 2015).
Endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
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 2015).
Equivalence (of phytosanitary terms)	The situation where, for a specified pest, different phytosanitary measures achieve a contracting party's appropriate level of protection (FAO 2015).
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2015).
Food plant	A plant on which adult psyllids feed, but do not breed and do not spend an extended period of time (e.g. diapause or winter season).
Fumigation	A method of pest control that completely fills an area with gaseous pesticides to suffocate or poison the pests within.
Host	An organism that harbours a parasite, mutual partner, or commensal partner, typically providing nourishment and shelter.
Host plant	A plant on which a psyllid species completes its immature to adult life cycle.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (FAO 2015).
Import permit	Official document authorising importation of a commodity in accordance with specified phytosanitary import requirements (FAO 2015).
Import risk analysis	An administrative process through which quarantine policy is developed or reviewed, incorporating risk assessment, risk management and risk

<b>Term or abbreviation</b>	<b>Definition</b>
	communication.
Infection	The internal 'endophytic' colonisation of a plant, or plant organ, and is generally associated with the development of disease symptoms as the integrity of cells and/or biological processes are disrupted.
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (FAO 2015).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (FAO 2015).
Intended use	Declared purpose for which plants, plant products, or other regulated articles are imported, produced or used (FAO 2015).
Interception (of a pest)	The detection of a pest during inspection or testing of an imported consignment (FAO 2015).
International Plant Protection Convention (IPPC)	A multilateral treaty for international cooperation in plant protection (WTO 2015)
International Standard for Phytosanitary Measures (ISPM)	An international standard adopted by the Conference of the Food and Agriculture Organization, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC (FAO 2015).
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO 2015).
Life cycle	Cyclical progression of stages in the growth and development of an organism (plant, animal, or pathogen) that occur between the appearance and reappearance of the same stage of the organism (Shurtleff & Averre 1997).
National Plant Protection Organization (NPPO)	Official service established by a government to discharge the functions specified by the IPPC (FAO 2015).
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 2015).
Overwintering or shelter plant	A plant on which adult psyllids overwinter, and on which they may feed.
Pathogen	A biological agent that can cause disease to its host.
Pathway	Any means that allows the entry or spread of a pest (FAO 2015).
Pest	Any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (FAO 2015).
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 2015).
Pest free area (PFA)	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 2015).
Pest free place of production	Place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period (FAO 2015).
Pest free production site	A defined portion of a place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production (FAO 2015).

<b>Term or abbreviation</b>	<b>Definition</b>
Pest risk analysis (PRA)	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 2015).
Pest risk assessment (for quarantine pests)	Evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences (FAO 2015).
Pest risk assessment (for regulated non-quarantine pests)	Evaluation of the probability that a pest in plants for planting affects the intended use of those plants with an economically unacceptable impact (FAO 2015).
Pest risk management (for quarantine pests)	Evaluation and selection of options to reduce the risk of introduction and spread of a pest (FAO 2015).
Pest risk management (for regulated non-quarantine pests)	Evaluation and selection of options to reduce the risk that a pest in plants for planting causes an economically unacceptable impact on the intended use of those plants (FAO 2015).
Pest status (in an area)	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information (FAO 2015).
Phytosanitary certificate	An official paper document or its official electronic equivalent, consistent with the model of certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements (FAO 2015).
Phytosanitary certification	Use of phytosanitary procedures leading to the issue of a phytosanitary certificate (FAO 2015).
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 2015).
Phytosanitary procedure	Any official method for implementing phytosanitary measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests (FAO 2015).
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 2015).
Polyphagous	Feeding on a relatively large number of hosts from different plant family and/or genera.
PRA area	Area in relation to which a pest risk analysis is conducted (FAO 2015).
Quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing or treatment (FAO 2015).
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 2015).
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 2015).
Regulated non-quarantine pest	A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (FAO 2015).
Restricted risk	Risk estimate with phytosanitary measure(s) applied.

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<b>Term or abbreviation</b>	<b>Definition</b>
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO 2015).
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
Stakeholders	Government agencies, individuals, community or industry groups or organizations, whether in Australia or overseas, including the proponent/applicant for a specific proposal, who have an interest in the policy issues.
Surveillance	An official process which collects and records data on pest occurrence or absence by surveying, monitoring or other procedures (FAO 2015).
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 protection against regulated pests.
The department	The Australian Government Department of Agriculture and Water Resources.
Tissue culture	The products of 'an in vitro technique of cultivating (propagating) cells, tissues, or organs in a sterile synthetic medium' (Shurtleff & Averre 1997); comprising plant cells, tissues or organs, sterile synthetic medium, and the vessel in which cells have been propagated.
Treatment	Official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalisation (FAO 2015).
Unrestricted risk	Unrestricted risk estimates apply in the absence of risk mitigation measures.
Viable	Alive, able to germinate or capable of growth.

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