

Threat specific contingency plan for huanglongbing and its vectors

Draft

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Further information

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1 Purpose and background of this contingency plan

This contingency plan provides background information on pest biologies and available control measures to assist with preparedness for an incursion into Australia of Huanglongbing (citrus greening) and its vectors. It provides guidelines and options for steps to be undertaken and considered when developing a Response Plan to this pest. Any Response Plan developed using information in whole or in part from this Contingency Plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

This contingency plan was developed for the Nursery & Garden Industry Australia (NGIA), and therefore is focused on production nurseries covered by this association. In the event of an incursion, operations not covered by the NGIA (e.g. retail outlets) will not be eligible for Owner Reimbursement Costs, as defined in the Emergency Plant Pest Response Deed, if affected by actions carried out under the Response Plan.

The information for this plan has been primarily obtained from the “Huanglongbing and its Vectors - A Pest Specific Contingency Plan for the Citrus and Nursery and Garden Industries”, prepared by GAC Beattie (University of Western Sydney) and Patricia Barkley (Citrus Australia Ltd.) in 2009. Since this plan is not yet endorsed by OCPPO and is not published online, it is subsequently referred to as Beattie and Barkley (unpublished). Modifications have been made to the plan to make the information relevant to an incursion of Huanglongbing in the Nursery and Garden Industry. Scenarios more relevant to citrus production areas are also covered.

2 Critical tasks

There are a number of areas which will require careful planning or implementation following the detection of Huanglongbing (HLB) or its vectors that are not covered in this contingency plan. These tasks include (but are not limited to):

1. Determine if the relevant pest is notifiable as per the state/s legislation.
2. If vectors have been detected, applying for emergency minor use permits for a broad range of products to allow a large rotation schedule. Suggestions have been provided in Appendix 9.
3. Establishing a diagnostic protocol for relevant pests. Protocols have been developed for HLB¹ and *Diaphorina citri*² but they remain incomplete. It is very important that the most efficient and reliable method for detecting HLB is used, and this changes on a regular basis.
4. Establish approved laboratories capable of diagnosing relevant organisms for each state.
5. Establish compliance guidelines, perhaps based on those used in Florida or California, to allow the relevant entities (production nurseries and/or orchards) to produce demonstrably pathogen and pest-free products within quarantine zones.
6. Since comprehensive lists of production nurseries across Australia do not exist, contact relevant state's NGI organisation to obtain a list of known production nurseries that produce relevant host plant species. Retail outlets should also be compiled and include regular, rigorous and independent auditing by appropriate authorities.

It is also recommended that a register be created for all production nurseries supplying citrus to commercial citrus growers prior to a detection of HLB, or its vectors, in Australia.

3 Australian nursery industry

The Australian nursery industry is a significant horticultural sector with a combined supply chain (production to retail/grower) valued at more than \$6 billion dollars annually. The industry employs approximately 45,000 people spread over more than 20,000 small to medium sized businesses, including production nurseries and retail outlets. The industry is located predominantly along the Australian coastline, and in major inland regions servicing urban and production horticulture.

¹ Deborah Hailstones and others (NSW Department of Primary Industries)

² Malik Malipatil and Linda Semeraro (Victorian Department of Primary Industries).

Nursery production adds significant value to Australia’s primary industry’s sector annually, contributing more than \$2 billion to the national economy. Nursery production is a highly diverse industry, providing a critical service to the broader horticultural sector, valued at \$14 billion within Australia (Table 1).

Table 1. Nursery production supply sectors within Australian horticulture

Production Nursery	Horticultural markets	Economic value
Container stock ³	Ornamental/urban horticulture	\$2 billion retail value
Foliage plants ³	Interior-scapes	\$87 million industry
Seedling stock ⁴	Vegetable growers	\$3.3 billion industry
Forestry stock ⁵	Plantation timber	\$1.7 billion industry
Fruit and nut tree stock ⁴	Orchardists (citrus, mango, etc) *	\$5.2 billion industry
Landscape stock ³	Domestic & commercial projects	\$2 billion industry
Plug and tube stock ⁶	Cut flower	\$319 million industry
Revegetation stock ¹	Farmers, government, landcare groups	\$109 million industry
Mine revegetation	Mine site rehabilitation	Value unknown

* The value of citrus tree stock, Australian native and exotic rutaceous host plants in production nurseries is unknown. Their distribution is extremely widespread, pervasive and in constant flux. Refer to point 6 of critical tasks above.

4 Eradication or containment decision matrix

Production nurseries are important as pathways for the potential entry and dissemination of huanglongbing (HLB) and its vectors. Nursery production systems are unique in that plants are sent far and wide and growth flushes occur frequently due to optimum growing conditions. Often HLB symptoms are not present or recognised because they resemble those of nutrient deficiency. Once psyllid vectors and/or bacterial pathogens are detected in a production nursery they will be subject to containment and/or eradication processes. Eradication of HLB and its vectors may be technically feasible if the disease and its vectors are detected while still contained within very small area/s prior to widespread distribution of infested plants to orchards and into the retail supply chain.

HLB, if introduced into Australia, will create significant and serious problems for citrus, and related species, production nurseries, significant ongoing problems for the citrus industry and potentially a disastrous environmental impact on rare and threatened native citrus species and other indigenous members of the family Rutaceae. However, the decision of whether or not to eradicate must be based solely on technical and economic feasibility.

The decision matrix to aid in the decision between eradication and containment is shown in Figure 1 and Table 2.

³ Data sourced from Market Monitor

⁴ Data sourced from Horticultural Handbook 2004

⁵ Data sourced from ABARE 2005

⁶ Data sourced from industry

Fig. 1. Decision outline for the response to an exotic pest incursion and a summary of the basis on which each decision could be made.

<p>Basis for technical feasibility:</p> <ul style="list-style-type: none"> ○ Early detection ○ Confined space/restricted area of dispersal ○ Known distribution of host plants ○ Effective, reliable, quick detection method ○ Support from industries, businesses and communities involved.
<p>Basis for economic feasibility:</p> <ul style="list-style-type: none"> ○ Value of crop destroyed by uncontrolled pest is more than cost of controlling the pest ○ Value of environmental amenity (native species lost) vs cost or loss of other amenity (loss of native insects due to spraying in native forests etc)
<p>Basis for quarantine containment:</p> <ul style="list-style-type: none"> ○ Legislation to create a pest quarantine area (PQA) ○ Resources to maintain the PQA, inspection points, staffing, detection equipment, diagnostics ○ Support of industry and community to make the PQA work
<p>Basis for destruction/control strategies required:</p> <ul style="list-style-type: none"> ○ How much destruction and or control measures are industry and individuals prepared to undertake? ○ What level of destruction is technically feasible? ○ Do the benefits of destruction outweigh the problems created?
<p>What would containment or ongoing management look like?</p> <ul style="list-style-type: none"> ○ Is containment feasible? ○ What would ongoing management really mean? ○ Many similar features to eradication, but at less intense / restrictive levels.

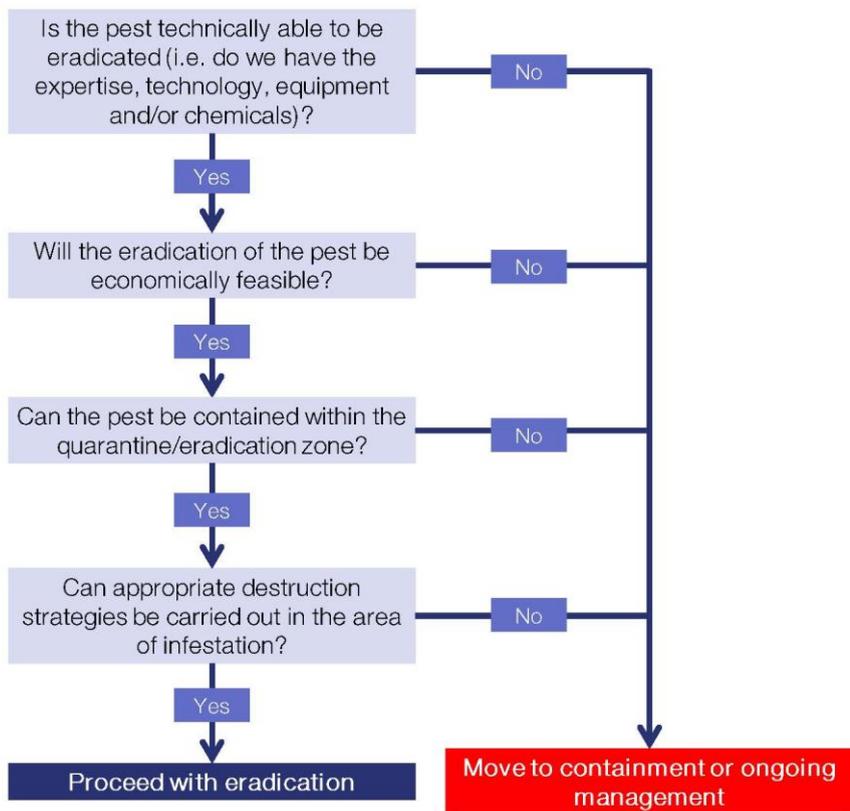


Table 2. Factors considered in determining whether eradication or alternative action will be taken for an EPP incident involving HLB and its vectors (modified from Appendix 12 of PLANTPLAN). More details are provided in sections 8 and 9

Factors that may favour eradication	Factors that may favour alternative action
<ul style="list-style-type: none"> • If initial detection area is relatively small and confined to one area, particularly if isolated. • If there are physical barriers and/or discontinuity of hosts between production districts. • Eradication is the best option if practically feasible as long-term management have not been successful overseas. • Populations of HLB vectors can only increase on actively growing host plants. This limits the capacity for growth considerably. • Pest biocontrol agents are not known or recorded in Australia. • If the level of pesticide resistance is relatively low (based on the origin of populations). • If trace back information indicates few opportunities for secondary spread. • Weather records show unfavourable conditions for pest development and spread. • Ease of access to outbreak site and accuracy of knowledge of alternate hosts. 	<ul style="list-style-type: none"> • If initial detection area is relatively large and or at multiple sites, particularly in built up suburban areas. • If the detection is in major areas of continuous production of host plants or has been distributed by production nurseries. • If vectors are present with HLB. • If trace back information indicates extensive opportunities for secondary spread. • If weather records show optimum conditions for pest development and spread. • If the outbreak area is in difficult terrain and/or there are problems accessing and locating host plants, particularly assessing all plants in urban environments including backyards, acreage properties, etc.

5 Pest information/status – Asiatic citrus psyllid

5.1 Pest details

Table 3. Asiatic vector – nomenclature (from Beattie and Barkley unpublished)

Common names	Asiatic citrus psyllid, Asian citrus psyllid, oriental citrus psyllid, citrus psylla
Scientific name	<i>Diaphorina citri</i> Kuwayama [Hemiptera: Sternorrhyncha: Psylloidea: Psyllidae]
Synonyms	<i>Euphalerus citri</i> Crawford

5.1.1 Background

The Asiatic citrus psyllid (*D. citri*) is a pest of citrus (including some native Australian citrus) and some ornamental plants. It damages plants directly through its feeding activities, and new shoot growth that is heavily infested by psyllids does not develop normally and is more susceptible to breaking off (Grafton-Cardwell et al. 2013). While direct damage is serious, there is even greater concern because the insect can transmit the lethal disease, huanglongbing (citrus greening).

Diaphorina citri is found throughout Asia, parts of North, South and Central America and some islands off Africa. Closer to Australia, it is found in Indonesia (including Papua), East Timor, north-western Papua New Guinea, Guam and American Samoa⁷ (Hall et al. 2013). The psyllid was recorded in the Northern Territory in 1915 following an incursion of citrus canker (Bellis et al. 2005). The psyllid was inadvertently eradicated during the campaign to eradicate citrus canker. *Diaphorina citri* has not been detected in Australia since.

⁷ http://www.pacificdisaster.net/pdnadmin/data/original/GUM_SPC_2008_PestAlertNo40.pdf; <http://www.hungrypests.com/the-threat/asian-citrus-psyllid.php>

5.1.2 Life cycle

Diaphorina citri has a simple life cycle that progresses from egg through five nymphal instars to the adult stage (Grafton-Cardwell et al. 2013). Adults usually feed on the underside of leaves, and will jump or fly a short distance when disturbed (Mead 1977). The life span of adults, which live for 1-2 months, is influenced by temperature and host plant (Liu & Tsai 2000). Adult females lay their eggs on the tips of growing shoots or in the crevices of unfolded “feather flush” leaves. The number of eggs laid depends on the host plant, but often ranges between about 400-850 over a female’s lifetime (Paiva & Parra 2012; Tsai & Liu 2000). At 25°C, eggs hatch in about 4 days (Tsai & Liu 2000) and nymphs feed exclusively on new growth. Nymphs move in a slow, steady manner and flick their abdomens upward when disturbed. The five nymphal stages look similar, but increase in size after each moult. Development from egg to adult takes about 9 to 36 days depending on host plant and temperature (Nava et al. 2007; Teck et al. 2011; Tsai & Liu 2000).

Adults *D. citri* are present year-round in southern Florida with peak psyllid populations in late spring, summer and autumn (Tsai et al. 2002). This coincides with new flush growth in *M. exotica* and grapefruit. There is evidence that the taxonomy of orange jasmine is somewhat misunderstood, often using *M. exotica* and *M. paniculata* synonymously. Morphological and molecular evidence suggests that they are separate species. Only *M. exotica* appears to be present in the Americas but at least two species are present in Australia (Holford et al. 2012). Orange jasmine is thought to serve as an alternative host for the psyllid when citrus is not in flush because of its more continuous flushing pattern; in Australia, this species is commonly known as mock orange (*M. paniculata*).

5.1.3 Dispersal

Movement of psyllid-infested plant material, including fruit and foliage, is the most common method of long distance spread of *D. citri*; it is a well established hitchhiker (Halbert et al. 2010) and can survive up to 10 days on detached stems and up to 20-30 days on detached stems with fruit (Hall & McCollum 2011). Ornamentals and food plants such as orange jasmine (*M. exotica*) and curry leaf (*Berbera koenigii*), respectively, have also been known to spread psyllids. Tropical storms and cyclones may lead to long distance spread of *D. citri*, as could illegal importation of host plants into Australia, including leaves such as kaffir lime, leaves or curry (*B. koenigii*) for cooking



Fig. 2. Adult and nymphal ACP feeding on citrus.



Fig. 3. Damage caused by large numbers of ACP (above) and black-sooty mould and waxy tubules on citrus leaves.

or citrus budwood. Adults are poor fliers, generally only flying about 30-100 m at a time, but long distance dispersal can occur with repeated short-distance flights and with wind assistance (Hall et al. 2013).

5.2 Affected hosts

5.2.1 Host range

Known hosts of *D. citri* are listed in Appendix 1, based on information from Halbert & Manjunath (2004) and Beattie and Barkley (Unpublished)⁸. All known breeding hosts of *D. citri* belong to the family Rutaceae, most within the Aurantioideae, but other non-breeding hosts are known from the family Moraceae and a legume (Thomas & De Leon 2011); it is not known whether such trees could be hosts for HLB. The suitability of any given host is influenced by cultivar, ambient temperature, nutrition, soil moisture, light (within shaded forests, on the edge of forests, under cloud or in sunny conditions), plant density (in sparsely spaced tropical forests or savannas, or in relatively dense monocultures), the nutritional value and the frequency of flush growth, and local biotypes of the psyllid. Young citrus trees that flush prolifically under ideal conditions are more suitable than older trees growing under identical conditions, and young and old trees grown under poor conditions. *Murraya exotica* growing in a shaded forest will be a less suitable host than the same plant growing as an ornamental in a well-maintained garden. Aubert (1988) noted that *D. citri* in Asia, in contrast to *T. erytraea* in Africa, is not able to build up massively on a wide range of alternative rutaceous host trees or shrubs in forests. Because new flush growth is required for proliferation of the psyllid, production nurseries are an ideal environment for *D. citri*.

From an eradication point of view, all citrus cultivars are considered hosts of the psyllid (e.g. orange, grapefruit, mandarin, tangelo, lemon, lime, kumquat, pomelo, trifoliolate orange and native *Citrus* species). However, the exact host plant species and variety can have a very large influence on colonisation and population build up. For example, varieties of *C. medica* 'Diamante citron' were considered very good hosts of *D. citri* whereas another variety (Indian citrus hybrid) of the same species was considered to be a very poor host. In general, *Citrus* spp., *Murraya* spp., *Berberis koenigii* and some *Microcitrus* varieties are very good hosts and other host plant species tend to be less preferred (Westbrook et al. 2011).

5.2.2 Current geographic distribution

As detailed in Beattie and Barkley (unpublished), *D. citri* occurs in the following regions and countries: Asia, the Arabian Peninsula (Saudi Arabia and Yemen), from Afghanistan through the Indian subcontinent, Southeast Asia and East Asia (the Ryukyu Archipelago and Kyushu in Japan, in China, particularly Taiwan and the coastal provinces of Guangxi, Guangdong, Fujian and Zhejiang), the Philippines and through the Indonesian archipelago to north eastern Papua New Guinea, Hawaii, Guam and Northern Mariana Islands, American Samoa. In the USA it is found in Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, South Carolina, and Texas. It is also in Puerto Rico, Bahamas, Cayman Islands, Cuba, Jamaica, Dominican Republic, Guadeloupe, Venezuela, Brazil, Paraguay, Uruguay, Argentina and, in the Indian Ocean, the Mascarenes (Mauritius and Réunion)⁹.

Diaphorina citri has been recorded in Australia. The record is based on a sample of 20 specimens collected at Stapleton (13°11' S, 131° 01' E), Northern Territory, by GF Hill in 1915 (specimens in British Museum of Natural History). The host of these specimens is unknown, but it was most probably a species or hybrid of non-native *Citrus*. It followed an incursion of citrus canker that is assumed to have been related to the introduction of *Citrus* from Asia to Darwin (then Palmerston: 12° 24', 130° 52'), about 100 km north of Stapleton, from Asia in the early 1900s (Bellis et al. 2005). *Diaphorina citri* has not been recorded since due to chance eradication during the 1916-1922 eradication campaign for citrus canker; all cultivated species and hybrids of *Citrus* trees north of 19°S were destroyed. It seems that the psyllid did not occur on naturalised or native *Movatifoliolata* (Bellis et al. 2005). Nor did it occur on any introduced citrus relatives such as *M. exotica*, *B. koenigii*, and *Clausena* spp., if they were present, or on *C. gracilis*, a species endemic to the northern region (north of about 13°S) of the Northern Territory and one that was not recorded until 1971.

⁸ A draft of 'Huanglongbing and its Vectors; A Pest Specific Contingency Plan for the Citrus and Nursery and Garden Industries' Version 2.

⁹ <http://www.hungrypests.com/the-threat/asian-citrus-psyllid.php>

5.2.3 Potential distribution in Australia

Climate modelling, and observations in Asia, suggest that the Asian citrus psyllid is likely to survive in much of Australia, including all citrus growing areas of Australia (Aurambout et al. 2009). Indications are that populations are likely to be highest in the Central Burnett, Riverland, Riverina and Murray Valley regions. *Diaphorina citri* will survive a wide range of temperature extremes from +45°C to – 7°C (Aubert 1990). Psyllid populations prefer low altitudes (< 800 m above sea level, depending on region) and high saturation deficits (warm to very hot climates with low relative humidity) (Beattie and Barkley, unpublished). Most production nurseries across Australia would provide the right microclimate to support proliferation of the psyllid.

5.2.4 Symptoms

Diaphorina citri directly damages citrus and closely related ornamentals (Halbert & Manjunath 2004). High numbers of nymphs distort the growth of leaves and stems and may cause death of new growth. Notching of leaves may also occur. Psyllids extract large quantities of sap from the plant as they feed and produce honeydew which can lead to the growth of black-sooty mould on plants.

5.3 Diagnostic information

Draft diagnostic protocols for the detection of *D. citri* are currently being developed by Malik Malipatil and Linda Semeraro (Victorian Department of Primary Industries).

Table 4. Identification of *D. citri* (from Hall et al. 2013)

Adults	Nymphs	Eggs
Small (3–4 mm), brownish, sap-sucking insects	Dull orange with red eyes.	Very small bright yellow-orange and almond-shaped.
The forewings are distinctively patterned with mottled brown patches.	Can secrete white, string-like waxy tubules.	Laid in groups on buds and young flush tips less than 10 mm long.
The abdomen has a pointed shape when viewed from above and may be gray/brown, blue/green or orange/yellow.	Can be difficult to see because they are small, flat, and close to the surface of twigs and leaves	
Distinctive feeding posture, with the head down, almost touching plant surface with body at 45° angle.	Mainly found on buds, leaves and stems of young flushing growth less than 50 mm long.	

5.4 Pathogen risk ratings and potential impacts

The Asiatic citrus psyllid (*D. citri*) can directly damage a large number of plants, mainly in the family Rutaceae (Appendix 1). High populations cause stunting and twisting of new growth and may result in black-sooty mould, leading to defoliation and a reduction in the value of nursery plants, particularly those that are favourable hosts, e.g. varieties of citrus and *M. exotica*. Besides through the illegal importation of plants, budwood and cuttings, winds created by tropical storms and cyclones may lead to the entry of infective psyllids into northern Australia. Thus the **entry potential** is rated medium-high. The psyllid will have a high **establishment potential** based on a history of establishment overseas, suitable climate and presence of alternative hosts. As populations of the psyllid can increase rapidly on active plant growth of its preferred hosts and the adult insects can be carried considerable distances in wind, it has a high **spread potential**. While infective psyllids carrying HLB will have high economic impact on the citrus industry and citrus production nurseries, the **economic impact** on the nursery industry in general is rated only as medium, as *Citrus* and other rutaceous plant species (e.g., *M. exotica*) comprise only a relatively small proportion of the total production nursery sector. Overall, *D. citri* is given a **medium priority** ranking as a production nursery pest, even though it is often ranked as a high priority citrus pest.

6 Pest information/status – African citrus psyllid

6.1 Pest details

Table 5. African vector – nomenclature (from OEPP/EPPO 2005; van den Berg 1990)

Common names	African citrus psyllid, citrus psylla, two spotted citrus psyllid
Scientific name	<i>Trioza erythrae</i> (del Guercio) [Hemiptera: Sternorrhyncha: Psylloidea: Triozidae]
Synonyms	<i>Aleurodes erythrae</i> del Guercio; <i>Spanioza eritreae</i> del Guercio; <i>Spanioza erythrae</i> (del Guercio); <i>Spanioza erythrae</i> del Guercio; <i>Spanioza merwei</i> (Petty); <i>Trioza citri</i> Laing; <i>Trioza erythrae</i> (Del Guercio); <i>Trioza merwei</i> Petty

6.1.1 Background

Like the Asiatic citrus psyllid (*D. citri*), the African citrus psyllid (*Trioza erythrae* del Guercio) is a sap-sucking insect that can also transmit HLB. Initially it was considered a minor pest of citrus, but was raised to a major citrus pest in the 1960's when it became known that it is a vector for HLB in South Africa (van den Berg 1990). Nymphs produce cup-shaped open galls on the under (abaxial) surfaces of leaves of *Citrus* and other Rutaceae on which they feed. In addition to galling, infestations can cause leaf distortion, curling, stunting and chlorosis (OEPP/EPPO 2005). Small flush points may be so densely packed with eggs that they may shrivel and fall off (van den Berg 1990). It can therefore be a serious pest. The African citrus psyllid does not occur in Australia (OEPP/EPPO 2005).

6.1.2 Life cycle

Trioza erythrae is confined to host plants in the family Rutaceae. Its native host in sub-Saharan Africa include *Clausena anisata* (horsewood), and *Vepris lanceolata* (white ironwood). On average, a female psyllid lays about 800 eggs over her lifetime (range 31-2542), which may be as long as 70-80 days (van den Berg 1990). However, adults overwinter on semi-dormant trees and may live for longer periods, although oviposition does not occur (van den Berg 1990). Eggs are lemon to dark yellow and are laid on the margins of new leaf growth and are anchored by short stalks, although they are occasionally laid on tender young thorns, flower buds or young lemon fruit (van den Berg 1990). Eggs hatch after 5-17 days, depending on temperature (van den Berg 1990). First instar nymphs feed on the underside of the leaves or soft stems where they begin to form open galls. The feeding of a large number of nymphs causes curling of the leaves, distortion of shoots and even cessation of growth. The nymphs moult five



Fig. 4. Nymphal *Trioza erythrae* on citrus. Photos by Peter Stephen, Citrus Research International, Bugwood.org.

times before becoming winged adults (van den Berg 1990). Nymphal development takes between 20 and 45 days depending on temperature and host plant (van den Berg 1990). Nymphs are pale yellow in colour with red eyespots, adults are pale green with black eyes upon emergence and progressively become dark brown (wings always remain transparent) (van den Berg 1990). Nymphs and adults do not produce honeydew, but instead form white granules which can appear as dust on highly infested plants (van den Berg 1990). In Africa, the number of generations of *T. erytreae* can be quite variable, being between 3 and 8 per year; populations are heavily dependent on climate and flushing rhythm (Catling 1972; Tamesse & Messi 2004).

6.1.3 Dispersal

While, *T. erytreae* is not a strong flier, adults do disperse short distances (50-200m) when forced to do so, i.e. when young leaves become scarce or when populations are very large (van den Berg et al. 1991a). Over short distances, adults fly well and jump when disturbed (OEPP/EPPO 2005). In the absence of any host plant, *T. erytreae* has been reported to fly up to 1.5 km with the aid of prevailing winds and can survive about 3-4 days (van den Berg & Deacon 1988). Van den Berg et al. (1991b) found that *T. erytreae* was most likely to be found in the northern and southern edge rows. Long-distance spread is most likely to occur via movement of plant material infested with psyllids, although tropical storms and cyclones could potentially carry them relatively long distances. Like the Asiatic citrus psyllid, the African citrus psyllid and HLB could be introduced into Australia through illegal imports of host plants (Bove 2006).

6.2 Affected hosts

6.2.1 Host range

Citrus cultivars are the main hosts of *T. erytreae* (e.g. orange, lemon, lime, grapefruit, mandarin, kumquat, tangelo, pomelo, native citrus and citrus rootstock). Native and exotic mock orange/orange jasmine (*Murraya* spp.), white ironwood (*Vepris lanceolata*), lime berry (*Triphasia trifolia*) and horsewood (*Clausena anisata*) are also hosts of the

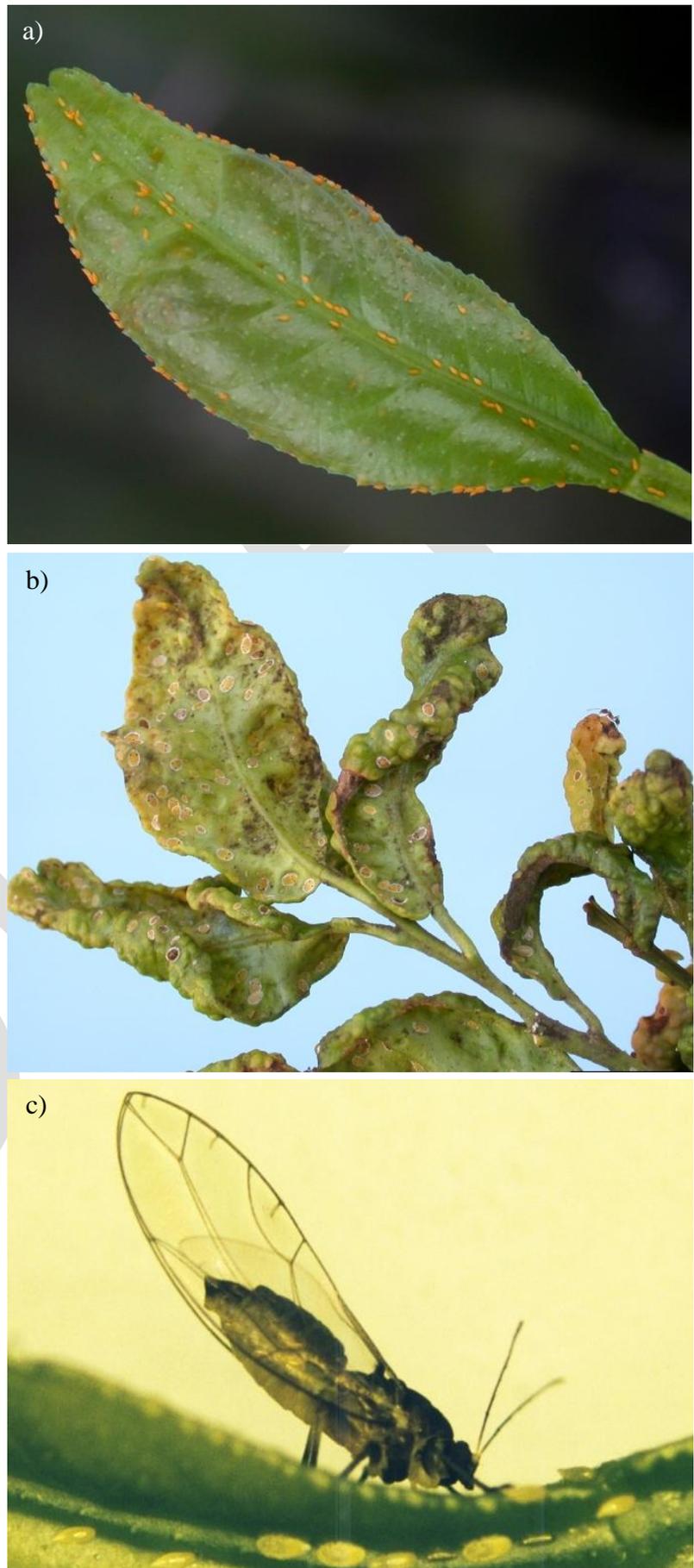


Fig. 5. Eggs (a), nymphs and pit galls (b) and an adult (c) *T. erytreae* on citrus. Photos by Peter Stephen (a and b) and SP van Vuuren (c), Citrus Research International, Bugwood.org.

African citrus psyllid. They can also feed on Cape chestnut (*Calodendrum capense*), orange-climber or forest-pepper (*Toddalia asiatica*) and small knobwood (*Zanthoxylum capense*) (Beattie & Barkley unpublished). Plant species with leaves that do not harden before nymphs eclose into adults are better hosts for *T. erytrae* because nymphs can not move to new growth once they have moulted into second instar nymphs (van den Berg 1990). Poorly nourished citrus leaves caused prolonged development, high rates of mortality and flattened nymphs of reduced size (Catling 1971). Studies overseas found that *T. erytrae* can transmit *Candidatus Liberibacter asiaticus* (Massonie et al. 1976).

6.2.2 Current geographic distribution

The African citrus psyllid prefers cool, moist climates and is sensitive to hot, dry weather (van den Berg 1999; van den Berg 1990). It occurs throughout sub-Saharan Africa, Saudi Arabia, Yemen, the Indian Ocean islands (Madagascar, Mauritius and Reunion), Atlantic Ocean islands (Saint Helena, Madeira, Porto Santo, Tenerife and Gomera, Canary, Madeira), Portugal and Spain (Canary islands, Palma de Mallorca and the mainland)¹⁰.

6.2.3 Potential distribution in Australia

Models estimating the potential distribution of *T. erytrae* in Australia have not been conducted. However, temperatures above 32°C with low relative humidity cause substantial mortality to eggs and first instar nymphs (Catling 1969); older stages have greater resistance to high temperatures but are still relatively susceptible (Catling 1969). Microclimate has been shown to significantly influence mortality of colonies. Highly shaded areas promoted *T. erytrae* survival such that numbers were higher in lower sections of tree canopies and near windbreaks; *T. erytrae* rarely will colonise small trees in hot regions (Catling 1969; van den Berg 1990). Furthermore, cool, humid seasons and mild summers produced higher populations than hot seasons (Catling 1969). *Trioza erytrae* prefers cool moist conditions with populations being consistently highest in cool, moist upland regions of its range (van den Berg 1990). As such, the climate in southern mainland Australia would be more suitable for its establishment and spread than in subtropical and tropical regions.

6.2.4 Symptoms

As African citrus psyllid nymphs feed, they cause distinct cup-shaped, open galls to form on leaves, particularly in the lower leaf surface of immature leaves. These are often visible as bumps on the upper leaf surface. They can cause severe leaf distortion, curling, stunting and leaf yellowing. The psyllids excrete pellets of honeydew that look like tiny, white eggs. The ground or vegetation under a badly infested tree can look like it has been dusted with white powder (the excreted pellets) (van den Berg 1990).

6.3 Diagnostic information

Draft diagnostic protocols for the detection of *T. erytrae* are currently being developed by Malik Malipatil and Linda Semeraro (Victorian Department of Primary Industries).

Table 6. Identification of *T. erytrae* (from OEPP/EPPO 2005)

Adults	Nymphs	Eggs
Adults are small, about 4 mm long. Males are smaller than females.	Nymphs are tiny (0.3–1.6 mm long). There are five nymphal instars.	Eggs are tiny, yellow or orange, cylindrical, and have an upturned, sharp point.
The abdomen is brown-grey, lighter underneath; the head is black.	Colour varies from yellow, olive-green to dark grey.	Each egg has a short stalk, which is inserted into the plant.
Males have an abdomen that ends in a blunt tip; the female's abdomen ends in a sharp point.	Nymphs are flat with a distinct marginal fringe of white, waxy filaments.	They are laid on leaf margins and along the midribs of young, tender, actively growing flush, and occasionally on flower buds and on young fruit.

¹⁰ <http://gd3.eppo.int/organism.php/TRIZER/distribution>

Adults	Nymphs	Eggs
The forewings are large and transparent with clearly defined veins.	Fifth instars have two pale brown spots on their abdomen.	
Adults have a distinctive feeding posture, with the head down, almost touching the plant surface, and the abdomen lifted at 35°.	They are mainly found on new flushing citrus growth.	
	The nymphs are largely sedentary (only first instars can move) and can form noticeable colonies on the underside of new leaves. They are sometimes found on the upper leaf surface if populations are high and overcrowded.	

6.4 Pathogen risk ratings and potential impacts

'*Ca. Liberibacter africanus*' and its vector *Trioza erytreae* represent a minor threat to the Australian nursery industry. If introduced their impact is likely to be greatest in the southern temperate regions rather than subtropical and tropical northern regions and will be perhaps limited by the absence of their principal alternative hosts. Both this form of the bacterium and its vector are heat sensitive. *T. erytreae* is also capable of transmitting *Ca. Liberibacter asiaticus* (Massonie et al. 1976) and can be assumed to be able to transmit *Ca. Liberibacter americanus*, although this has yet to be confirmed.

As both pathogen and vector are more restricted geographically and distant from Australia, their most likely entry pathway would be the illegal importation of budwood or cuttings of citrus or ornamental hosts. There is unlikely to be wind assisted movement into Australia of infective *T. erytreae*. They would therefore have a **medium entry potential**. **Establishment potential** would be high in southern temperate regions, particularly at higher altitudes. Based on the history of HLB in South Africa, **spread potential** would be medium. The disease would have a medium **economic impact** on production nurseries in southern regions, given that citrus only makes up a small portion of the total nursery production industry. Considering overall impact on the Australian nursery industry, it would have a **low priority** ranking as a production nursery pest.

7 Pest information/status – Huanglongbing (HLB)

7.1 Pest details

Table 7. Huanglongbing (HLB) – nomenclature (from Beattie and Barkley unpublished)

Common names	Huanglongbing (official common name), citrus greening (informal common name), likubin or decline (Taiwan), leaf mottling (Philippines), citrus dieback (India), citrus vein-phloem degeneration (Indonesia), citrus greening, yellow branch or blotchy-mottle (South Africa)
Scientific name	' <i>Candidatus Liberibacter asiaticus</i> ' (Asiatic form), ' <i>Ca. L. africanus</i> ' (African form) and ' <i>Ca. L. americanus</i> ' (South American form)
Synonyms	' <i>Ca. Liberobacter asiaticus</i> ' and ' <i>Ca. Liberobacter asiaticum</i> ' ' <i>Ca. Liberobacter africanus</i> ' and ' <i>Ca. Liberobacter africanum</i> '
Abbreviations	' <i>Ca. L. asiaticus</i> ' is known as Las ' <i>Ca. L. africanus</i> ' is known as Laf ' <i>Ca. L. americanus</i> ' is known as Lam

7.1.1 Background

Huanglongbing (HLB), which can be translated as "yellow shoot disease", is a bacterial disease that is lethal to *Citrus* and some other plants in the family Rutaceae. In citrus, HLB causes branch or leaf yellowing, leaf drop, misshapen fruit, fruit drop and tree decline. It is a serious threat to citrus production areas worldwide. HLB

probably originated in China or India (Gottwald 2010), and was given its name because of its characteristic symptom, a yellowing of some new shoots in an otherwise green canopy (Bove 2006). HLB occurs in many parts of the world, including Asia, parts of North, Central and South America, and Africa. Closer to Australia, it is found in Indonesia (from Sumatra to Papua), East Timor and Papua New Guinea. HLB and the psyllid insects that transmit it are not known to occur in Australia, but their potential for introduction is acute.

Huanglongbing is caused by at least three putative species of the genus '*Candidatus Liberibacter*', named according to the region from which they have originated; '*Ca. L. asiaticus*' from Asia (Las), '*Ca. L. africanus*' from Africa (Laf) and '*Ca. L. americanus*' from South America (Lam). They are all phloem-limited, uncultured bacteria that live in the host plant's phloem, where they impede the movement of nutrients. All species and cultivars of citrus are affected, such as orange, grapefruit, mandarin, tangelo, kumquat, lemon, lime, pomelo, trifoliate orange and tangelo, native citrus and orange jasmine (*Murraya* spp.). Trees may die within 5-8 years of infection.

The main psyllid vectors are the Asiatic citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erythrae*) (Bove 2006), however, two additional psyllids may be vectors, *Cacophylla (Psylla) citrisuga* and *Diaphorina communis*. The later two psyllids have been found positive with Las but require further testing to determine if they can transmit the disease to healthy plants (Cen et al. 2012; Donovan et al. 2012). It is likely that all psyllids that transmit one strain of HLB also have the capacity to transmit all strains they come into contact; *D. citri* has been shown transmit Las, Laf and Lam (Bove 2006; Teixeira et al. 2005) and *T. erythrae* can transmit Las and Laf (Massonie et al. 1976).

Long-distance spread can occur by the movement of HLB-infected citrus plants, budwood and cuttings or by the movement of plant material (including foliage and fruit) infested with HLB-infected citrus psyllids (Hall & McCollum 2011). Movement of other host plants such as orange jasmine (*M. exotica*) and curry leaf (*Bergera koenigii*) also pose a risk of introducing HLB-infected Asiatic citrus psyllids. Tropical storms and cyclones may also lead to long-distance spread of infected Asiatic citrus psyllids from Indonesia (especially Timor, Papua and adjacent islands) and Papua New Guinea to northern Australia.

7.1.2 Life cycle

The bacteria live in the host's phloem and transmission occurs when infected plant material comes in close contact with uninfected plant material, whether that be by grafting or even contact with parasitic plants, e.g. dodder (*Cuscuta indecora*) (Bove 2006; Hartung et al. 2010b). As a result, any cuttings and marcots made from infected plants will also be infected. However, HLB is not present in infected plants in a uniform fashion. Some plants grafted from HLB infected trees onto uninfected rootstock may remain free of HLB (Gottwald 2010).



Fig. 5. Symptoms of HLB on citrus. Photos by Andrew Miles (DAFF)

7.1.3 Dispersal

Long-distance spread can occur by the movement of HLB-infected planting material (plants, budwood, cuttings and rootstocks) of infected hosts. Also, movement of plant material infested with HLB-infected citrus psyllids, for example fruit and or leaves may spread HLB; *D. citri* can survive up to 20-30 days on picked fruits and or leaves (Hall & McCollum 2011). Most short-distance spread occurs by the insect vectors, the Asiatic citrus psyllid and the African citrus psyllid. Movement of other host plants such as orange jasmine (*Murraya* spp.) and curry leaf (*Berbera koenigii*) also pose a risk of introducing HLB-infected Asiatic citrus psyllids. Long distance movement of Asiatic citrus psyllid and Las have been documented on wind currents across the American Everglades (Hall et al. 2013), thus spread from Indonesia and Papua New Guinea to northern Australia is a high risk. Illegally imported host and non-host foliage may also spread psyllids infected with HLB, as indicated by interceptions into California from outside continental USA¹¹. Curry leaves are considered high risk for transporting *D. citri* and foliage from various herbs have also been detected with the psyllids, sometimes infected with HLB¹¹. Evidence indicates that HLB is not seed transmitted, despite early claims that it might be (Albrecht & Bowman 2009; Capoor et al. 1974; Hartung et al. 2010a).

The rate at which HLB spreads is significantly influenced by the extent of the inoculum reservoir, local vector populations, the age of host plants when first infected and many environmental factors (Gottwald 2010). In areas where host plants are not managed well (where vectors and infected plants are not controlled) in young plants, 50% or more of plants may become infected in 3-5 years. In older plants, under the same scenario, it may take 5 or more years (Gottwald 2010).

Overseas, where HLB and its vectors are both present, HLB is more prevalent at the edges of citrus orchards and along roads, canals, ponds and other geographical features within citrus plantations (Gottwald 2010). This is probably due to the behaviour of citrus psyllids preferring such sites, and therefore increasing the rate of transmission to such trees. However, some psyllids still move into the centre of citrus blocks.

7.2 Affected hosts

7.2.1 Host range

All species and cultivars of citrus are affected, such as orange, grapefruit, mandarin, tangelo, kumquat, lemon, lime, pomelo, trifoliolate orange and tangelo, and native citrus. Mock orange or orange jasmine (*Murraya* spp.) can also be a host plant. See Appendix 3 for an up-to-date list of known host plants.

7.2.2 Current geographic distribution

The distribution of HLB is expanding (Beattie and Barkley, unpublished)¹². It is present in the following locations:

- South and Southeast Asia (from the Indian subcontinent to the Philippines, Indonesia, East Timor and Japan), New Guinea (Papua and Papua New Guinea).
- The Arabian Peninsula, Saudi Arabia where 'Ca. L. asiaticus' is transmitted by *D. citri*, in Yemen where a 'heat-sensitive form' is transmitted by *T. erytraeae*, and north of the Saudi Arabia/Yemen border, where both vectors are associated with both 'Ca. L. asiaticus' and 'Ca.L. Africanus'. It is also present in Iran and Pakistan.
- In Africa, caused by 'Ca. L. africanus' and transmitted by *T. erytraeae*.
- In Mauritius and Réunion caused by 'Ca. L. asiaticus' and 'Ca. L. africanus' and most probably transmitted by both vectors.

¹¹ www.cdfa.ca.gov/phpps/ar/2009_ar/PHPPS_AR_2009_PE.pdf;
http://www.sdcountry.ca.gov/reusable_components/images/awm/Docs/ipd_asian_citrus_psyllid.pdf

¹² <http://www.cabi.org/isc/?compid=5&dsid=16567&loadmodule=datasheet&page=481&site=144>

- In the United States of America (Florida, Georgia and Louisiana, Texas, California) and Puerto Rico, caused by 'Ca. L. asiaticus' and transmitted by *D. citri*.
- In Mexico, Cuba and Central America
- In Brazil caused by 'Ca. L. asiaticus' and 'Ca. L. americanus' and transmitted by *D. citri*. HLB has also been detected in Argentina¹³.

A sub-species, 'Ca. L. africanus subsp. capensis', occurs in Cape chestnut (*Calodendrum capense* Thunb. [Rutaceae: Rutoideae]), an ornamental trees in southern Africa (Garnier et al. 2000), but is not associated with HLB in commercial citrus in South Africa (Pietersen et al. 2010). It seems likely that other sub-species will be identified over time in different geographic regions on additional host plant species. None of the diseases have been recorded in Australia (Bove 2006).

7.2.3 Potential distribution in Australia

Most production nurseries are located near towns and cities along the Australian coastline (within about 100km of the coast) with some in major inland areas servicing urban centres and horticultural industries. All these production nurseries are in areas that have climates suitable for 'Ca. Liberibacter asiaticus' and *D. citri*, whereas 'Ca. Liberibacter africanus' and its vector *T. erythrae* are likely to have the greatest impact on production nurseries in more southern temperate regions of Australia.

7.2.4 Symptoms

The Asiatic form, 'Ca. L. asiaticus', is the most geographically widespread, severe and is typically vectored by the Asian citrus psyllid. The African form, 'Ca.L. Africanus', is less severe, more restricted geographically and is considered heat-sensitive (Bove 2006). Symptoms are produced under somewhat moist, cool conditions between 20 and 27°C and at higher elevations (900 m) (Garnier and Bové 1993). In 2004, a third form was identified in Brazil: 'Ca. L. americanus' (Teixeira et al. 2005). So far, the form recorded in South America is only known in Brazil and is less heat tolerant than 'Ca. L. asiaticus' (Lopes et al. 2009). In this case the bacterium is vectored by *D. citri* (Bove 2006).

Key HLB symptoms in infected, commonly cultivated, citrus plants are (Bove 2006):

- leaves with asymmetric, sometimes dull, blotchy-mottling that crosses leaf veins
- mottled or complete yellowing of leaves and growing shoots (yellow shoots standing out from an otherwise normally green canopy)
- small, upright, thickened, chlorotic leaves (sometimes resembling mineral deficiencies, particularly Zn)
- flushing of severely greened trees out of phase with healthy trees
- out of season flowering and fruiting on infected branches
- leaf drop and dieback of branches
- small, lopsided fruit with small, dark, aborted seeds
- unevenly coloured mature fruit
- premature and excessive fruit drop
- bitter, insipid tasting fruit.

A number of common citrus pests, diseases and disorders in Australia cause symptoms that can be confused with huanglongbing. In particular, zinc deficiency and Australian citrus dieback are easily confused. Leaf mottling caused by zinc deficiency superficially resembles that caused by HLB, leaf mottling by zinc deficiency

¹³ <http://www.senasa.gov.ar/contenido.php?to=n&in=11&ino=11&io=21289>

runs along veins, whereas HLB mottling crosses leaf veins. Australian Citrus dieback (ACD), also a bacterial disease, causes similar asymmetric leaf mottling to HLB and reduces the size of fruit. However, ACD does not cause fruit deformity and does not cause fruit to become very bitter. In addition, ACD does not cause trees to die (Broadbent 2000; Garnier & Bove 2000). Foliar symptoms of HLB on *Murraya* spp. are similar to that on citrus.

7.3 Diagnostic information

Diagnosis of HLB symptoms can be confirmed using appropriate molecular methods. The current diagnostic approach is to use conventional or real-time PCR (Polymerase Chain Reaction) (Li et al. 2008). These tests can determine the presence or absence of HLB when symptoms are present and are capable of distinguishing between the Asiatic and African forms of the disease. In Queensland, excised “midribs” from leaves which have been dried over calcium chloride for 1-2 weeks, are used to analyse for the pathogen in samples collected during routine surveys. Robust diagnostic methods are still required to determine the presence or absence of HLB in the absence of symptoms, which can take months, or even years, to develop (Gottwald 2010)¹⁴.

Draft diagnostic protocols for the detection of HLB are currently being developed by Deborah Hailstones (NSW Department of Primary Industries) and others. The PaDIL biosecurity toolbox has detailed notes on molecular methods to detect HLB¹⁵. Since HLB is being researched by a many laboratories around the world, it is likely that new methods of detection will be developed overtime, including commercial available kits, e.g. those at www.plantprint.net.

7.4 Pathogen risk ratings and potential impacts

HLB is rated as having a medium-high **entry potential** with most likely entry pathways being via illegal importation of budwood and cuttings of citrus and other host plants and wind assisted movement of infected *D. citri* into northern Australia. The latter may occur in air movement associated with cyclones and severe tropical storms from the Indonesian Archipelago (particularly Timor and Papua, and adjacent islands). Availability of new citrus varieties overseas increases the risk of illegal introduction of planting material from South Africa, Florida and Asia.

Vectors, with or without the pathogen, are likely to enter Australia through human migration, travel (yachts, boats, aircraft) and trade in plants and fruit, especially in the Torres Strait. As adult psyllids are strongly attracted to light they may enter in commercial or military aircraft. Seed transmission of HLB is now considered to be negligible but concern still remains, indicating that caution should be taken when importing seeds from hosts of HLB (Hartung et al. 2010a). Entry may also occur with legally infected or infested plant material which has been inadequately treated or inspected (Beattie and Barkley unpublished).

Based on suitable climate, its history of successful establishment overseas and disease symptoms not easily identified in the field, **establishment potential** is considered to be high. The presence and distribution of alternative hosts of *D. citri* in northern Australia will favour establishment. These include commercial *Citrus* species and *Murraya* species in native or naturalised vegetation, orchards, production nurseries, parks and home gardens. Native *Citrus* is continuous around the Australian coastline, *Murraya* is common in gardens and the native *M. ovatifoliolata* var. *ovatifoliolata* occurs in coastal monsoon vine-thickets. Thus **spread potential** will be high. There are no known natural enemies of the vectors in Australia and there is the possibility of HLB transmission by other phloem feeders.

If HLB enters without its vectors, infected host plants in natural habitats and gardens will probably die and it will be a manageable threat. If HLB becomes widely distributed through the production chain and distributed in budwood the threat would be substantially higher. If the vector or vector/ pathogen establishes in a production nursery growing *Citrus* and *Murraya* the **economic impact** on these host plants will be high. Considering that these host plants make up only a small portion of the nursery production industry, the **economic impact** will only be medium for the nursery industry. Plants will have to be protected in the production nursery by applying synthetic pesticides, cultural management practices and growing in insect-proof protected cropping structures.

It is accepted that the impact on citrus specific production nurseries is likely to be high due to the ‘monoculture’ of cropping only citrus varieties and the defined market for these crops. Mitigation of the pest threat will require these production nurseries to invest in high cost infrastructure and systems if they are to remain in production for the long term.

¹⁴ http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/cg-nprg.pdf page 3-11

¹⁵ <http://old.padil.gov.au/pbt/index.php?q=node/28&pbtID=94>

Besides its impact on the Australian citrus industry and on rare and endangered native *Citrus* spp., an incursion of the vector or vector/pathogen will seriously affect the production nursery trade in citrus trees and other host plants in Rutaceae such as orange jasmine (*Murraya* spp.) and curry leaf (*Bergera koenigii*) through cost of screenhouse construction, regulated movement of nursery stock, increased pest control, insect monitoring, pathogen testing, etc.

8 Pest management

8.1 Response checklist

The following checklist (Table 8) provides a summary of generic requirements to be identified and implemented within a Response Plan.

Table 8. Checklist of requirements to be identified in a Response Plan

Checklist item	Further information
Destruction methods for plant material, soil and disposable items	Section 8.1.1
Disposal procedures	Section 8.1.4; 8.1.5
Quarantine restrictions and movement controls	Section 8.3
Decontamination and Hygiene Requirements When Conducting a Survey	Section 8.1.2; 8.5
Diagnostic protocols and laboratories	Appendix 6
Trace back and trace forward procedures	Section 8.6
Protocols for delimiting, intensive and ongoing surveillance	Section 8.6
Zoning	Section 8.4
Reporting and communication strategy	Appendix 7

For a range of procedures relevant to the response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia, 2010). Additional information is also provided by Merriman and McKirdy (2005) in the Technical Guidelines for Development of Pest Specific Response Plans (www.planthealthaustralia.com.au/go/phau/biosecurity/general-biosecurity-information). However, specific recommendations have been made below in response to the detection and containment of HLB and or its vectors. Limited on-going pest management strategies for HLB and its vectors are suggested for businesses that would like to continue to trade within quarantine zones which are also relevant to their management under containment scenarios.

8.2 Overview of surveillance and summary of logic following a detection

Host plants

No one recommendation can be made regarding how pest surveillance and eradication will take place following a detection of HLB and or its vectors in Australia. A wide variety of factors will influence management decisions, the most important of which is the location of the detection in relation to host plants (i.e. production nurseries, retail outlets, citrus producers and rural and residential centres, particularly large cities). An important course of action following the detection of an emergency plant pest (EPP) is to identify businesses and regions that could be associated with the movement of citrus and other host plant species to new areas, i.e. trace-forwards and trace-backs. Such high risk locations include, but are not limited to:

- Production nurseries supplying retail outlets
- Production nurseries supplying commercial citrus growers
- Retail outlets
- Produce and garden outlets
- Local markets

- Backyard (hobby) growers
- Residential home gardens
- Public gardens
- Proximity to commercial citrus orchards or abandoned citrus orchards

Since there are currently no lists of these high risk locations in Australia, it is recommended that the response team first determine the locations within close proximity of the detection site (see below for recommended distances under different scenarios). Consultation with the local Nursery and Garden IDO for the area may assist in the rapid preparation of a list of relevant businesses (Appendix 10). Presence of production and retail outlets supplying relevant susceptible plant species within this zone represent one of the highest risks to long distance spread of HLB and its vectors. Production nurseries pose a greater risk as they are more likely to be trading with interstate retailers and/or orchards. Such businesses should be notified immediately and supply of host plants to areas outside the quarantine zone, without a certificate verifying area freedom issued by a biosecurity inspector, must cease immediately. Posters alerting members of the public should be posted at all local retail outlets to increase awareness of the biosecurity threat. Furthermore, production and retail businesses within the zone should begin conducting surveillance for detected species, described in detail below, keeping written records that could support area freedom.

Trace-forward and trace-back plant movements should be established from all production nursery businesses connected to the original detection. Movement of stock from within the quarantine zone during the previous six months should be considered as a minimum trace-forward. Trace-back movements should also be identified; paying most attention to stock that was received within six months of the detection. For both trace-forward and trace-back plant movements the critical period could be longer than six months for HLB, owing to the long period of time required for symptoms to appear. This period of time should, of course, be modified based on the individual circumstances of the detection. However, an initial period of six months is suggested as a suitable compromise between scientific rigour and the practicalities of responding to a detection, and is the standard taken by the USDA (USDA 2012).

Insect vectors

Where *D. citri* or *T. erytrae* is found in a production nursery that is in close proximity to potential host trees and shrubs, regularly inspect nearby hosts for signs of psyllid infestation, i.e. at least once per month. Possible host plants include members of the family Rutaceae which includes the genera *Citrus* (cultivated and native), *Clausena*, *Glycomis*, *Micromellum*, *Murraya* and *Bergera*, *Choisya* and *Calodendrum*.

Populations of *D. citri* or *T. erytrae* will vary considerably with flushing rhythms and extent of flushing. In general in Australia, adult citrus trees have a spring flush, multiple summer flushes and an autumn flush (i.e. all year round except winter). However, this can be influenced by cultivar, climate and growing situation, e.g. nitrogen application and water availability. For nursery stock in warmer regions of Australia (e.g. Queensland), citrus plants can flush almost continuously, although at reduced levels in winter periods. All life stages (eggs, nymphs and adults) may be present on the new growth or shoot tips, while eggs are only laid on growing tips and nymphs cannot survive on old foliage. Surveillance should be higher when trees are flushing. As the insect feeds, it injects toxic saliva into the plant that causes the young shoots to be malformed, twisted, curled or laterally notched. In severe cases there will be leaf abscission and shoot tips will die. Sooty mould fungi may grow on the honeydew secreted by the psyllids. These symptoms may aid workers performing surveys to more easily detect the presence of psyllids.

Other vectors

Nursery personnel and personnel conducting surveys should avoid moving infested material between production nurseries. Every precaution should be taken when working in an infested area, including disinfecting implements, washing down vehicles and changing clothing and footwear (see section 9.1.2). Transfer of HLB infected material can occur very easily when host plant material is non-symptomatic and insect vectors are not always obviously present (eggs are very small and inconspicuous). Therefore, extreme care must be taken to ensure that material is free of all pests prior to moving it to a new location.

The following sections provide details on early warning surveillance following a detection of HLB and or its vectors under different scenarios. Be aware that these scenarios are necessarily general, will not cover every situation and will require modification and tailoring to the specific situation following an actual detection. However, they do provide practical guidance on the general processes involved, and may assist in the preparation of detailed survey procedures.

8.2.1 Summary of important biological information for planning delimiting surveys

When developing surveys for the presence and /or distribution of HLB and its vectors, the following characteristics provide the basic biological knowledge needed to underpin the survey strategy:

- The presence of HLB in the absence of its vectors will probably lead to the death of the infected plant within 5-8 years. However, the disease does have a long, latent, symptomless period (several months, but sometimes up to 1-2 years).
- Symptoms alone should not be used to diagnose a plant as being infected with HLB, because they can resemble other Australian diseases or abiotic disorders. Likewise, the lack of symptoms can not be used to determine the absence of HLB. Furthermore, molecular tests are not currently sensitive enough to detect HLB in symptomless plants.
- Most known hosts of the Asiatic citrus psyllid (*D. citri*) belong to the family Rutaceae.
- Host plants that flush prolifically are a more suitable host plant for the psyllid than those which flush infrequently. Therefore, *Murraya* spp. must be considered high risk host plants in Australia, even if they are not as good a host as citrus varieties.
- Significant areas of Australia have favourable climatic conditions for *D. citri* establishment and spread. African citrus psyllid is more suited to southern, cooler regions of Australia.
- *D. citri* is a short-distance flier, but may cover longer distances with multiple short bouts of flying. They are more likely to be found about 1-2 m above ground than above or below these heights (Chiaradia et al. 2008). Therefore, sticky traps amongst host plants should be placed within these heights.

8.2.2 Surveys for early detection of an incursion in a production nursery

The first line of defence against HLB and its vectors is their exclusion. However, early detection is critical, particularly in heavily built-up, residential areas.

Greatest entry risks come from the illegal introduction of plants, budwood or cuttings or from the legal importation of host plants that have not been adequately inspected or treated (including internet sales). In addition, tropical storms and cyclones could lead to the long distance spread of *D. citri* from Indonesia and Papua New Guinea to northern Australia. Awareness information should be targeted at managers of production nurseries and commercial citrus growers to ensure that they are familiar with the risks of importing illegal planting material. Material should also describe the legal method by which plants can be introduced and educate growers how to identify and inspect for the presence of the vector and disease symptoms. Of great importance is that there are no known psyllids that use citrus as a host in Australia. Therefore any psyllid found on citrus in Australia likely represents an EPP. Factsheets have been developed by a number of Australian agencies and could be used as part of awareness material ¹⁶.

Frequent (weekly) monitoring of production systems are recommended and staff must be familiar with the symptoms of HLB and able to identify psyllids and the damage they cause. They must be familiar with the life cycle of the psyllid, its characteristic honey dew and the deformed flush growth symptoms it can cause. Appropriately kept records demonstrating the absence of psyllids and HLB symptoms, may assist in providing evidence of area freedom and in initial delimiting surveys.

Surveys conducted by production nursery managers differ from that of regulatory authorities, therefore both types are considered below.

¹⁶ http://www.ngia.com.au/Category?Action=View&Category_id=682&Highlight1=fact%20sheet&Highlight2=fact%20sheet
http://www.dpi.nsw.gov.au/_data/assets/pdf_file/0005/432626/Exotic-Pest-Alert-Asiatic-citrus-psyllid.pdf
http://www.daff.qld.gov.au/_data/assets/pdf_file/0012/73110/Citrus-Asian-citrus-psyllid.pdf
http://www.daff.qld.gov.au/_data/assets/pdf_file/0017/56240/Citrus-African-citrus-psyllid.pdf
http://www.daff.qld.gov.au/_data/assets/pdf_file/0003/54183/Citrus-HLB-similar-symptoms.pdf

8.2.2.1 Monitoring by production nursery managers

Systematic, regular and careful inspection of nursery plants and propagated material for signs of pests and disease, should be the basis of all monitoring processes. A range of detection methods should be implemented and performed by production nursery managers or consultants on behalf of the grower/owner.

- Yellow sticky traps should be placed about 1 m above the ground in areas where host plants of HLB are present, particularly all citrus and *M. exotica*, at a rate of not less than 1 trap per 50 m² of growing area; relatively large numbers of traps are required to detect low populations of citrus psyllids (Hall 2009). Note that standard recommendations as per BioSecure HACCP are 1 trap per 200m²; the above recommendation is given specifically for nursery producers in quarantine zones monitoring for citrus psyllids. Traps should be inspected on a weekly basis as a part of routine pest surveillance in the production nursery and also serve as a method to detect citrus psyllids. These guidelines may be met by Interstate Certification Accreditation (ICA) requirements.
- A representative sample of all host plant species should be visually inspected on a weekly basis for all insects and disease symptoms (weekly crop monitoring plan). The presence of HLB vectors should be easily identified due to their characteristic nature, e.g. angled feeding habit, distinctive curly, solid honeydew or pitting/deformation of new growth. Mother plants should be monitored for the presence of disease symptoms on at least a weekly basis. Symptoms consistent with HLB should be reported to the emergency plant pest hotline (1800 084 881).
- Plant beating or sweep netting (described in more detail below) can be used to rapidly monitor a large number of plants for a wide variety of insect pests. The presence of any psyllids on citrus should be followed by an immediate report of the pest to their local biosecurity organisation.
- The NGIA Nursery Production Farm Management System provides greater detail on crop monitoring, site surveillance and consignment inspections under the BioSecure HACCP program.

Plant beating involves moving through a crop and gently but firmly hit foliage against a beating tray (which can be a folder, bucket or plastic plate). The beating tray should be a single colour; white or black is preferable as this will make moving organisms more visible. Beating plants is a relatively efficient way of monitoring for insects and mites that are visible to the naked eye and can be knocked from plants, including psyllids, herbivorous and predatory mites, aphids, whitefly adults, thrips, lady beetles, small caterpillars and a variety of other insects.

Sweep netting is similar except it requires the use of an insect net being swept across foliage. Move the net side to side while walking around trees or down rows. Similar to plant beating, insects and mites that can be dislodged from a plant will be collected in the net.

8.2.2.2 Surveillance by regulatory authorities

Biosecurity staff should regularly survey host plant species of HLB and its vectors in all areas of Australia as part of their regular surveillance. However, sole reliance on this surveillance is probably insufficient. HLB, *D. citri* and *T. erytrae* may not have been listed as a notifiable species in every state and territory. High risk areas include northern Australia (in response to increased risk of movement of plants into Australia via South-Pacific islands and southeast Asia) and within and near commercial citrus growing areas. Inspection of mother stock plants in production nurseries represents a more strategic, risk based method of conducting surveillance, as these plants are more likely to be consistently flushing and their progeny will be sent to many regions across Australia. Retail outlets and production nurseries are infrequently monitored as part regular biosecurity surveillance. Therefore, business owners are left responsible for reporting suspect EPPs. In relation to HLB and its vectors, surveys should be completed from spring to autumn and any time there is active plant growth.

Visual inspections and plant beating should be used at each site, ensuring that a significant portion of the crop is sampled. Where the growing area of relevant host plants is small, it may be possible to inspect nearly all plants. In other cases, where large numbers of plants are being grown over vast areas, it may be possible only to inspect 10-20% at any one time. In this situation, different plants should be inspected each time. Priority for inspection should be given to plants which have abnormal growth. Mother stock plants should be inspected wherever possible.

All psyllids collected from citrus, or other HLB host plant species, should be collected into 96-100% ethanol. In the event that a highly suspect specimen has been found, it should be reported to the local biosecurity organisation so that they can take a sample and send it to the state's relevant expert (see Appendix 6) for identification. If positively identified as an EPP, the specimen diagnosis will need to be confirmed by a second expert, usually in another laboratory/state from the original diagnosis. Specimens will be tested for the

presence of HLB using PCR techniques (See list of pathology experts below). Plants exhibiting symptoms consistent with HLB require diagnostic testing using real-time PCR techniques. Businesses with plants found with highly suspicious symptoms (be they HLB or psyllids) should be asked not to move plants until results indicate that they are free of EPPs.

8.2.3 Delimiting surveys in the event of an incursion response

In the event of an incursion by *D. citri*, *T. erytreae* and/or HLB, delimiting surveys are essential to inform the decision making process. The specific actions taken and the exact size of the quarantine area will vary depending upon the species detected and their location; particularly in relation to highly built-up residential areas, commercial citrus growing areas, production nurseries and retail outlets and other potential sources of spread (e.g. weekend markets). A number of different scenarios (based on that from Beattie and Barkley (unpublished)) are subsequently considered in this document, and describe courses of action for each case.

In every case, all plants with symptoms consistent with HLB should be tested for HLB using real-time PCR. Psyllid vectors found on plants should also be tested for presence of HLB using PCR techniques. HLB can be found in psyllids more accurately than from non-symptomatic plants. Psyllids collected and found to be positive for HLB should be assumed to indicate that the tree from which they were collected is also positive for HLB, particularly if the individual was a nymph. For this reason, it is important that samples of psyllids only be taken from individual plants and that each plant is marked in such a way that it can be identified at a later date. Furthermore, if the detection is associated with HLB and a vector, every tree found with psyllids should be treated as though it is also infested with HLB, even if symptoms of HLB are not yet present.

8.2.3.1 Example 1. Isolated areas are where the distribution of *Citrus* and closely related species are generally sparse in native vegetation, parks and home gardens and dense residential areas do not occur within a 5 km radius. This example is characterised by a single point of detection, in a location where there is an absence of businesses (or other entities, e.g. home fruit seller) that move either citrus fruit, or whole plants, out of the region (i.e. interstate or other geographic regions for distribution).

Establish a 5 km quarantine zone around the point of detection. In the event that HLB was confirmed and vectors were not associated with the detection, destroy all host plants found to be positive for HLB within 11 km of HLB positive plants. All plants should be tagged and recorded for GPS data for ease of returning to and destroying plants if necessary. Presence or absence of active growth should be recorded. If active growth is present on plants, and psyllid vectors are not found in the area, continue monitoring plants every three months for at least three years, or destroy all host plants within 1 km of HLB positive plants. HLB does not move by itself therefore trace-backs are extremely important for determining how the infestation originated, and dictate what further actions need to be taken.

In the event that psyllid vectors were detected (in the presence or absence of HLB), delimiting surveys should be conducted on a wider scale to determine if eradication is feasible. Such surveys could start about 2-5km from the detection site and widened if psyllids are detected. If psyllids are not detected around the 2km radius ring, all host plants should be destroyed within this area. If psyllids are found at distances equal to or greater than 5km, feasibility of eradication will need to be assessed and action taken accordingly.

Actively growing plants within the quarantine zone should be surveyed as a priority. Surveys should be completed throughout the quarantine zone, starting at the original detection site.

Note: Trace-forward to sites outside the quarantine area may be required if a weekend market occurs within the quarantine zone.

8.2.3.2 Example 2. An isolated area at a citrus orchard, but not a production nursery. This example is characterised by a lack of dense residential zones within 15 km (reflecting the greater capacity for large populations of psyllids to develop), few *Citrus* and related plant species occurring in native vegetation, and parks and home gardens at sparse rates with a single point of detection.

Establish a 15 km radius quarantine zone. In the event that HLB was detected and vectors were not associated with the detection, determine the likely ability for HLB to have been spread within the orchard, i.e. if any grafting has occurred on site. Survey every plant within 1 km of the initial point of detection. Surveys must focus on collecting samples for molecular diagnostic tests of symptomatic plants because testing of non-symptomatic plants is not reliable. If over 20% of plants are found to be positive for HLB (as confirmed by real-time PCR), destroy all plants in the orchard block (block is considered loosely here as being a logical unit with regard to

relevant factors, e.g. trees purchased from one supplier on sample date). If fewer than 20% of the total number of plants that were originally in the block are found to be positive for HLB, destroy all plants with symptoms and survey all remaining plants every three months. Ensure that trace-backs surveys include the supplier/s of infected plants. Survey plants in native environments within 1 km of the orchard. Presence of HLB in such plants indicates that a vector is likely to be present (even if it has not been found at the original point of detection).

In the event that psyllid vectors are detected in the absence of HLB and the vector is not found outside of the citrus orchard, all host plants should undergo appropriate insecticide treatment to eradicate the psyllids (the exact treatment will vary depending upon the registration status of specific insecticides at the time of the incursion; however see Appendix 9 for general insecticide advice). All host plants outside of the citrus orchard, within a 2 km radius of the orchard including plants introduced at weekend markets, should also be destroyed prior to the destruction of the orchard to create a buffer-zone over which psyllids are unlikely to migrate. Surveys outside of the infested orchard, within the quarantine zone, should be conducted monthly while host plants are actively growing. Host plants on transport routes (i.e. roads, railway lines), along which citrus fruit or host plants have travelled to outside markets, should also be surveyed for the presence of psyllids.

In the event that psyllid vectors are detected in the presence of HLB, detailed surveys should be completed outside the orchard of initial detection with a 2 km radius. If there is no indication that vectors and HLB have moved beyond a 2 km radius, destroy plants within this area to create a buffer zone that reduces the potential spread of citrus psyllids. In the event that the distribution has spread beyond 2 km of the initial detection point, eradication will need to be considered based on the exact circumstances and cannot be prescribed here. If HLB and its vectors are contained to a single orchard, the orchard should be sprayed to eradicate psyllids, particularly adults. All host plants should be destroyed within 2 km of the orchard, including those in the orchard, starting distally and working towards the centre. Host plants within the quarantine zone should be surveyed on a monthly basis during active growth phases.

Note: Trace-forward to sites outside the quarantine are may be required if a weekend market occurs within the quarantine zone.

8.2.3.3 Example 3. In an isolated area at a production nursery. This example is characterised by a lack of dense residential zones within 10 km (reflecting a moderate capacity for large populations of psyllids to develop), *Citrus* and related species occurring in native vegetation, parks and home gardens at sparse rates and a single point of detection.

Establish a quarantine zone in a 5 km radius from the original point of detection. While surveys of the production nursery and immediately surrounding regions are important and will influence decisions on the feasibility of eradication, of greater initial importance are long distance trace-forwards and trace-backs (off-site mother stock sources). Since production nurseries are often sending stock to retailers and/or citrus growers which may be a long way from their farm. Establishing the presence or absence of HLB and or its vectors at these sites is extremely important for determining whether the incursion is eradicable. Trace-back of scion or bud wood material (either on-farm sources or off-farm sources which can be from interstate) used in the production cycle will also be critical in the overall delimitation of the incursion. In general, production nurseries should be monitored for pests and disease on a weekly basis and have the capacity to detect HLB and its vectors.

In the event that HLB is detected in the absence of its vectors at a production nursery, and it is still believed to be an isolated point of detection, all host plants that potentially could have come in contact with HLB should be destroyed. Trace-backs surveys may help to determine the source of infection and the length of time it has been present. Hosts of HLB that have been sent to retailers within the past 6 months (or another time frame based on trace-back information) and/or citrus growers should also be destroyed. If plants that are likely to be infected with HLB have been sent to many disparate areas and sold to numerous members of the public, eradication should be reconsidered. Destruction of all infected mother stock plants and management of the production of mother stock material could potentially eradicate HLB from Australia in the absence of its vector. Rigorous, specific regulations would be required to deal with this situation, which are beyond the scope of this document. Control and containment regulations used in the USA could provide a good starting point for Australia in this scenario.

In the event that HLB vectors are detected in the absence of HLB (as determined by PCR of relatively large numbers of psyllids), all host plants at the production nursery should be treated with insecticide (the exact treatment will vary depending upon the registration status of specific insecticides at the time of the incursion; however see Appendix 9 for general insecticide advice). All sites that have received host plants may have also had vectors present should be monitored and managed appropriately (i.e. proactive management of relevant host plants to reduce the risk of establishment of vector species). Ideally, host plants in the surrounding areas

should also be monitored (the exact range is not prescribed as purchased plants infested with the vector will appear in the community at random).

In the event that HLB and its vectors are detected at a production nursery, all host plants should be destroyed, including mother stock plants, located on-farm and off-farm, used by the production nursery within the past two years. Host plants of the vector should not be grown at the production nursery for at least 1 month. All host plants from the initial site of infestation that have been sent to a retailer and/or citrus grower within the last 6 months (or another time frame dictated by trace-back information) should also be destroyed if at all possible. Destruction of these plants should be completed hygienically, i.e. sprayed with an insecticide to reduce the likelihood of vectors moving to nearby plants. Surveys around trace-forward sites should be conducted as per paragraph above. If multiple disparate trace-forward sites exist, eradication is unlikely, but should still be considered. The extent of the area over which HLB and its vectors are found will dictate the feasibility of eradication.

Note: Trace-forward to sites outside the quarantine area may be required if a weekend market occurs within the quarantine zone.

8.2.3.4 Example 4. In a dense residential area. This example is characterised by detection of HLB and/or its vectors in a dense residential area in a backyard/home garden host plant (e.g. on *Citrus* or *Murraya*). The detection may be in close proximity to production nurseries growing citrus, and host plants are common within surrounding residential homes, gardens, public parks and weekend markets. However, the detection is restricted to a small detection point (<200 m²).

Establish a quarantine zone in a 2km radius around the initial site of detection. It will be very important to quickly identify businesses which may sell or distribute host plant species within a 2 km radius of the detection point. These businesses represent the greatest risk of long distance spread of HLB and its vectors. Delimiting surveys should be conducted within 500 m of the detection area initially, and expanded if high numbers of host plants are found. If weekend markets are conducted within the quarantine zone trace-forward to production site will form part of any delimiting surveillance.

In the event that HLB is detected, but not its vectors, trace-back surveys will be very important to identify the source of the infestation. Relevant actions will need to be taken accordingly. All host plants with symptoms of HLB will be destroyed and all host plants within 500 m of the detection area will be monitored every three months or destroyed, whichever is more practically feasible. In addition, surveys within 5 km of the detection site should be conducted at least twice per year until eradication is complete or containment/transition to management alternatives are implemented. Retail outlets and production nurseries selling *Citrus* (commercial and native species and varieties), and species of *Murraya* (e.g., orange jasmine), *Berberis* (e.g. curry leaf) and *Clausena* (e.g. huangpi or wampee), weekend markets and roadside stalls within 5 km of the detection site should be surveyed every three months. Particular attention should be given to mother stock plants at production nurseries within 5 km of the original detection. Under this scenario, eradication should be considered.

If HLB is detected, in the absence of its vectors, at retail outlets or production nurseries in dense residential areas and there is reason to believe that plants have only been distributed to local areas, a containment zone will have to be created based on the information available and feasibility of eradication. Movement of all host plant species from within the zone being sent out of the zone should not occur unless local area/property freedom can be established (e.g. by using proven pathogen free mother stock plants). Under this scenario short term eradication is probably not feasible, but may be possible if the infestation has actually been contained to the quarantine area. A massive public awareness campaign and removal of all infested host plants within the quarantine zone may eventually eradicate HLB within 8-10 years. In addition, all HLB host plants being used as mother stock plants should be destroyed and replaced with pathogen free mother stock plants (mother stock plants could be located within the quarantine zone or in another area). If plants believed to be positive with HLB have been spread to other regions of Australia, these trace-forwards and quarantines should be established in a relevant fashion.

If vectors of HLB are detected in the absence of HLB (by PCR testing of relatively large numbers of the vector) on residential premises an initial intensive survey should be conducted within a radius of 200-400 m of the detection. Assuming the infestation is contained, all relevant host plants should be treated with an insecticide (the exact treatment cannot be prescribed here as only one product has a registration which could legally be used to treat citrus psyllids; however see Appendix 9 for insecticide advice) within 400m of the detection area.

Retail outlets, weekend markets and production nurseries within a 5 km radius of the initial detection should be surveyed. Ongoing monitoring by production nursery staff (as per section 8.2.2.1) is required with written records inspected every 4 months. Retail outlets, weekend markets and production nurseries within 1 km of the

detection area should also be required to treat all plants with an insecticide (the exact treatment cannot be prescribed here as only one product has a registration which could legally be used to treat citrus psyllids; however see Appendix 9 for insecticide advice). If a vector is detected at a retail outlet and it is likely that plant material was only sold locally, a quarantine zone based on available information should be created to prevent movement of all host plant species from within the zone being sent out of the zone unless local area/property freedom can be established (e.g. growing plants in an insect-free protected structure, with positive pressure, rigorous management and monitoring plan).

If the vector is detected in multiple sites around the initial detection area, surveys should be conducted over a larger area; i.e. in about a 2 km radius around the detection area. If further detections are made the delimitation will have to be widened further and feasibility of eradication rapidly declines. If the host plant is not actively growing, it will only be possible to detect adults; ideally, survey actively growing plants as this is more likely to lead to an accurate positive or negative result.

If HLB and its vectors are detected on residential premises, an initial intensive survey should be conducted within a radius of 200-400 m of the detection and less intensive survey 2 km from the detection area. If surveys indicate the infestation has been contained to a small area, all host plants of HLB and its vector should be destroyed within 400 m of the detection area. Eradication may be possible.

If the vector is relatively widely distributed then a larger containment zone should be established,, preventing movement of all host plant species from within the zone being sent out of the zone unless local area/property freedom can be established (e.g. growing proven pathogen free mother stock plants and propagated material in an insect-free protected structure, with positive pressure, rigorous management and monitoring plan). The exact size of the quarantine will have to be determined depending upon the extent of the infestation. Under this scenario, containment is probably the best case scenario.

Note: Trace-forward to sites outside the quarantine are may be required if a weekend market occurs within the quarantine zone.

8.2.3.5 Other points to consider

These examples are not exhaustive but provide a framework for many scenarios that might occur. In each case public outreach should play a major role, particularly if the detection is in a dense residential area. In the case of an incursion there should be a focus on homeowners and public officials in the infested areas, retail outlets, garden clubs and traditional and social media. It will be necessary to sit down with journalists from print and electronic media to reach the public and create a sense of urgency for targeted audiences to inspect citrus and ornamental trees. Keep messages simple and tied to driving specific behaviors (e.g. check your citrus trees and report suspicious symptoms; don't move host plant material). Communications should drive people to a website to tell their story. Public officials should be engaged early on for their support and extend the message to their constituents. Facebook, Twitter and one-on-one discussion forums can also be used to good effect.

The exact response will depend on where incursions are detected. Regions where commercial orchards and production nurseries occur require the most rigorous delimiting surveys to determine the extent of an affected area, or areas. The first point of detection may not be the initial location of the incursion. Trace-back and trace-forward analyses must therefore be undertaken to determine the source of an incursion and to identify other premises or locations that may have been exposed because of their proximity to the point of initial detection, movement of infected planting material or fruit, or prevailing winds carrying adult psyllids. Surveyors should be trained to ensure that they are capable of recognising HLB symptoms (differentiating them from other citrus diseases and disorders), stages in the life cycle of the psyllid vector, direct damage and honeydew associated with the vector and damage, and that they have sound knowledge of the biology of the pathogen and the vector. They must also be able to identify hosts of the pathogen and the vector.

The extent of infection/infestation in a production nursery, property, or within a region will depend on the initial population size and whether conditions have been favourable for the pest to spread from the initial foci or for infection of HLB to occur. Sampling should be based on the origin of the initial suspect sample(s). Factors to consider for decision making will be:

- The proximity of other susceptible host plants to the initial infestation source including crops in the production nursery or on the property with the initial detection and those on neighbouring properties in gardens, parks, native vegetation, weekend markets, etc.
- Movement of machinery, vehicles, personnel and plants that have been in the infested area or in close proximity to the infestation site and around the infested area.

- A possible link to the recent importation of plant material from other regions should be considered via budwood, rootstocks, trees and whether any other crops have been propagated from the same source and/or distributed from the affected production nursery.
- If the vector is present, the lifecycle and spread potential of the vector will need to be considered.
- Meteorological records of wind movement should also be considered over the time period the infestation is suspected to have been present in Australia.

In addition, observation of lop-sided poorly coloured fruit with a bitter taste, may alert authorities to the presence of HLB. Trace-back to the farm of origin would be essential.

Make sure you stay flexible as the situation changes or as more information is made available.

8.2.4 Collection and treatment of samples and survey procedures

8.2.4.1 How to survey individual plants for HLB and its vectors

Surveying a tree is simple but requires careful examination. Trained surveillance teams should inspect plants on foot looking for HLB symptoms. The most reliable symptom of HLB is asymmetrical, small, bitter tasting fruit. Inspect foliage for blotchy mottling of leaves, corking of leaf veins, leaves with yellow veins, yellow shoots and short upright and bunched growth. Younger plants show symptoms of HLB sooner after infection than older plants, especially if budwood or mother stock plants are infected. If plants are more than 2 m tall, a tractor mounted platform should be used to supplement ground surveys for HLB.

Inspect new growth for presence of citrus psyllids using the following methods:

- Visual inspections for adults on mature leaves, particularly on the underside of leaves in between flush cycles and in regions with distinct winters.
- Visual inspections for eggs and nymphs on flush growth from 5 mm to 50 mm long, particularly in spring, within 14 days of buds opening. Detection of eggs will require at least a x10 hand lens. Honeydew may appear like 'snow' but is blown off plants readily in the wind.
- Beating foliage, particularly young flush growth, to dislodge adults into a 300 mm-diameter pan containing a shallow amount of mineral oil (to prevent adults from flying away).
- Use of yellow sticky traps to trap flying adults.

In addition, Asiatic citrus psyllid causes leaf notching and deformity, which is pronounced on fully expanded, older leaves. Presence of sooty mould and waxy tubules on foliage may provide clues for the presence of *D. citri*. Pit galls on young leaves indicate that African citrus psyllid could be present. Sweep netting can also be used to sample for psyllids on taller trees. Note also that citrus psyllids show a pronounced edge effect, particularly in citrus orchards.

On residential properties, all host plants should be surveyed, walking around each tree to inspect all areas for HLB and its vectors. At production nurseries and retail outlets, inspect all citrus and other host plants. Plant beating of young foliage may increase the speed and detection of psyllids. At commercial citrus orchards inspect as many plants as possible, moving up and down each row. Ensure that at least 20% of the orchard is sampled for very large farms (1 in 5 rows). If any plants are found to be positive for HLB, survey all plants in the orchard.

Make sure you have obtained permission to enter the property before starting a survey. Enquire as to what pesticides have been applied recently as this may affect the abundance of insect vectors as well as preclude safe entry to a orchard. Particular care must be given when collecting individual specimens, to prevent contamination of samples and to maintain confidence in the sample from collection to its final result by a diagnostician. Collectors must exercise extreme care in handling specimens to ensure that hands, tools, and other collection supplies do not become a source of contamination between samples and particularly between individual sites. Other exotic pests and pathogens may be present if the infestation was via illegal importation of plant material; most countries with 'Ca. L. spp.' also have citrus canker.

Wash bottles containing soap and disinfectant must be provided to disinfect hands and small tools and articles. Special care must be taken with pruning instruments and other items used for sampling. Care must also be taken with larger articles such as shoes, clothing and vehicles, to be certain that

these do not become a means of contamination or spread of any pathogens that may be present (e.g. citrus canker or soil borne diseases). See QDPI&F Work Instruction ST-W-006¹⁷.

8.2.4.2 Data collection

During surveys, data must be recorded according to guidelines set out in ISPM No. 9 (1998), especially at the site of detection or occurrence. Data collected during a preliminary investigation should be used to estimate the potential for spread, the anticipated rate of spread and to identify endangered areas. Information gathered and recorded on the Survey Form should include (Beattie and Barkley unpublished):

- Geographical location using GPS; see QDPI&F Work Instruction ST-W-001¹⁸.
- Hosts infested at the site including age, variety/clone, rootstock, phenology.
- Extent and impact of damage and level of pest prevalence.
- How the pest was detected and identified.
- Recent imports of plants or plant products including nursery stock movements.
- History of the pest on the property or in the area.
- Movement of people, products, equipment and conveyances.
- Mechanism of spread within the area, including likely source of inoculum (infected trees, infected budwood, spread by storm etc.).
- Climatic events and soil conditions including storms and prevailing wind directions.
- Condition of infested plants, including age of plant parts affected (spring flush/autumn flush etc).
- Orchard management including method of irrigation, cover crops and spray programs.

8.2.4.3 Sampling procedures for symptomatic plant material

Sample collection procedures are heavily based on Irey (2007) and Beattie and Barkley (unpublished). Each sample collected should contain green twigs of 15 cm to 20 cm long with about 20 leaves, preferably with the petiole still attached and with good recognisable symptoms. Molecular diagnostic tests are not reliable unless symptoms are present, therefore all material collected must be symptomatic (NAPPO 2012). All insect vectors should be removed from branches, if present. This can be done in a number of ways but may include lightly brushing, rinsing and drying the branch.

The total number of HLB symptomatic plants must be recorded. Samples should, if possible, be photographed before they are removed from plants. The entire plant should also be photographed and include the affected area of plant. Other photographs should include a perspective of where the symptomatic plant is located with respect to other plants. A minimum of 3 photographs per symptomatic plant is recommended, unless several plants in a block are symptomatic, in which case fewer photographs may be acceptable.

All plants from which a sample has been taken must be marked with the sample ID number so that it can later be destroyed if it is shown to be positive for HLB. This is a simple process in backyards and most production nurseries. If very large numbers of plants are present it may be necessary to mark the ends of rows so that it is easier to re-locate the symptomatic tree, e.g. in commercial orchards.

1. Stems should contain green twigs of 15 to 20 cm long with a minimum of 20 leaves. 1-4 representative symptomatic stems can be collected per tree. Fruit may be left on the branch or trimmed. If trimmed, the peduncle should be left on the stem (trim as close to the button of the fruit as possible). If a variety of symptoms are present, the preferred samples (in order of preference) would be:
 - a. branches with mottled leaves;
 - b. branches that contain shoots that are almost entirely yellow;
 - c. branches that have leaves with yellow veins;
 - d. branches with leaves that have either green islands on a yellow background or yellow islands on a green background;
 - e. branches with nutrient deficiencies that have a 'rabbit's ear' appearance (small, upright leaves);
 - f. branches with leaves that show chlorosis and 'vein-corking';
 - g. branches with Zn and/or Fe deficiencies that are not related to blight or other known causes.
2. Place leaf and stem samples with paper towels in two resealable plastic bags, one bag inside another larger bag.
3. Express air from bags.

¹⁷ Telford G. 2007 Decontamination and Hygiene Requirements When Conducting a Survey for Citrus Canker. QDPI&F Work Instruction ST-W-006.

¹⁸ Telford G. 2007. Global Position System (GPS) Unit Use and Calibration. QDPI&F Work Instruction ST-W-001.

4. Seal bags.
5. Record sample identification number and date of collection on bag.
6. Keep samples cool, but not frozen (in an ice chest).
7. Send samples such that they will definitely arrive at the destination and not remain in transit over a weekend.

To prevent contamination of samples, hands and small implements should be disinfected between samples. Pruning shears and other items used to cut host plants should be disinfected between cuts and prior to use on a new property. This procedure will avoid spreading citrus exocortis or other citrus viroids. These citrus pathogens can be carried on the cutting surfaces of pruning shears, knives, and other implements used for cutting and pruning operations. Making a cut on an infected tree is sufficient to contaminate the cutting tool; subsequent cuts on other trees will introduce the viroid and infect the tree. A 10% solution of common household bleach (sodium hypochlorite with 1-2% available chlorine) should be used to inactivate citrus viroids.

8.2.4.4 Sampling procedures for psyllids

Detection of HLB from psyllids is much more reliable than from plant material, even when they are collected from non-symptomatic trees. Trees from which psyllids have been collected and tested positive for HLB should also be considered infected with HLB and destroyed. Ideally, psyllids should be identified to ensure that it is a citrus psyllid prior to diagnostic tests for HLB. However, there are no known psyllid species that attack citrus in Australia, nor other host plants of HLB. Therefore it is reasonable to assume that any psyllid found on relevant plant species are a potential vector of HLB. Nevertheless, a sample should be sent the relevant experienced authority, if in doubt.

1. For psyllid identification, place the insects in a leak-proof vial containing 70% ethyl alcohol.
2. For PCR analysis for 'Ca. Liberibacter' (or phytoplasma), place the insects in a leak-proof vial containing 95% ethyl alcohol. The proper alcohol concentration is important for effective PCR analysis. If psyllids have been dead for more than 2 days prior to being preserved in 95% ethanol the results of PCR analysis may not be accurate.
3. Submit at least 5 insects; 10-20 being more are preferable.
4. Write the sample ID number in pencil on a label inserted into the vial.

Some overnight carriers will not accept specimens preserved in alcohol due to regulations on air transport of flammable materials. Ground transportation may be required. Samples can be sent via Australia Post if appropriate guidelines are followed, i.e. CASA special provision A180 – non-infectious specimens supported or preserved in alcohol/formaldehyde¹⁹. These guidelines indicate that 70% ethanol can be sent in vials (less than 30mL) if they are double-bagged and both bags are heat sealed. The second bag must have absorbent material that would absorb all liquid in case of a spill. The outer packaging must contain cushioning and be marked appropriately – refer to guidelines in the event that any changes have occurred.

8.2.4.5 Assigned laboratories

Samples should be sent to an approved laboratory in the affected State/Territory and to the designated diagnostic laboratory. At this time, such laboratories do not exist and would need to be rapidly identified following an incursion, although appendix 6 lists a number of diagnostic laboratories which may have the capacity. Transportation and laboratory facilities must be organised so that samples are processed within 24 h to 48 h of collection. A number of support laboratories are also required to provide confirmation of diagnostic tests from the assigned laboratory when there is an excess of material to be tested, when specific techniques and expertise is required or when skilled personnel at the assigned laboratory are not available.

8.2.4.6 Criteria for the determination of a positive or negative results

The following criteria are used by the Florida Southern Gardens HLB Diagnostic Laboratory (http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf).

Test results fall in one of three categories:

1. HLB positive – test results indicate that 'Ca. Liberibacter sp.' was detected in the sample,
2. No HLB found – test results did not indicate that 'Ca. Liberibacter' was present in the sample, or
3. An inconclusive test result (re-testing should be done).

¹⁹ http://www.casa.gov.au/scripts/nc.dll?WCMS:STANDARD:1334079869:pc=PC_100324

No testing procedure is 100% accurate. If a sample is designated as 'no HLB found', this does not mean that the tree/plant from which the sample was taken is disease-free – it means that no 'Ca. Liberibacter' was detected in the sample. This could be because:

- No 'Ca. Liberibacter' was present,
- 'Ca. Liberibacter' was present, but below the limit of detection,
- 'Ca. Liberibacter' was present, but the sample was inadequate for testing (sample was in poor condition, wrong tissue type was sampled, wrong sampling time), or
- The test failed.

In Australia, similar symptoms to those of HLB are caused by Australian citrus dieback (Broadbent 2000) and sometimes by severe stem pitting strains of CTV in grapefruit. A phytoplasma has been associated with Australian citrus dieback (Davis et al. 1997). It is recommended that a phytoplasma probe be used on all negative suspect samples.

8.2.4.7 Notification of symptoms

If a grower, consultant, or other person detects a suspect case of HLB, *D. citri* or *T. erytraeae*, this person should ring the Exotic Plant Pest Hotline 1800 084 881 which will notify a representative of the Plant Health Manager of the State or Territory in which the samples were found.

If a diagnostic laboratory receives samples of diseased material suspected of being HLB or citrus psyllids, notification to the Plant Health Manager of the State or Territory in which the samples were found should include a full description of the symptoms or pest and the reasons why these are suspected to be those of HLB or a citrus psyllid.

8.3 Availability of control methods

HLB can only be controlled when coordinated efforts are made to eliminate infected plants, psyllid vectors are managed and only pathogen/vector-free nursery plants are distributed. Overseas, production nurseries have been able to produce healthy citrus trees using insect-free protected cropping structures and pathogen free motherstock. The main problem is that once those plants are not housed in a protected structure they can be colonised by citrus psyllids and infected with HLB quickly. Control of HLB is generally difficult if inoculum sources are widespread and psyllid populations well established; complete management of HLB has not yet been achieved overseas. However, below are best practice management guidelines which at least reduce the likelihood that a plant becomes infected with HLB. Control measures will be drastically different when HLB is present in absence of its psyllid vectors. In any case, the below management techniques will aid in the containment of HLB and its vectors to facilitate the development of longer term management strategies.

8.3.1 Procedures for management of HLB (vector absent)

- Use of pathogen-free rootstocks and scions in lieu of potentially infected budwood, scions, and marcotts.
- All citrus budwood source trees and mother plants shall be tested and indexed on a regular basis to ensure the disease-free status of citrus material.
- Frequent monitoring for HLB symptoms. The number of times may differ for different situations. For example, old plants take longer to exhibit symptoms, therefore sampling every 2 months is sufficient. Whereas, young plants exhibit symptoms more quickly, therefore monthly sampling is more appropriate.
- Security and sanitation measures to prevent pest or disease introductions.
- Mandatory area-wide management practices in commercial citrus orchards (this will be difficult in Australia where orchard sizes are much smaller than in Brazil or Florida and where not all growers will diligently and effectively apply vector control measures).
- Prompt destruction (cutting down and poisoning of roots) of diseased trees within orchards, home-gardens, nursery retailers and production nurseries.
- Mandatory and immediate destruction of all trees in a block (be it a citrus orchard or production nursery) should the percentage of HLB infected trees reach or exceed 10% of trees within an interval of 12 months.

- Destruction of mother stock plants from which produced plants subsequently have been confirmed with HLB.
- Destruction of abandoned orchards²⁰.
- Mandatory removal of alternative hosts of the vectors and liberibacters, particularly species of *Murraya*, *Berbera* and *Clausena*, within close proximity (2 km) of orchards²¹.
- Register all citrus production nurseries that supply plants to citrus orchards, Australia wide.
- Register all sellers of citrus and other HLB host plant species within quarantine zones to facilitate more efficient monitoring of high risk spread pathways.
- Education of farm and production nursery and retail outlet personnel; pesticide manufacturers, distributors and retailers, consultants, technical advisors, and scouts; research, quarantine, regulatory and advisory staff within government departments.
- Training and education of employees to recognise exotic diseases. Employer to maintain a log of training.
- Set requirements for all commercial citrus growers to source certified citrus budwood and seed as free from HLB. All citrus plants must remain free from arthropods and diseases of concern to maintain certification. The integrity of the structures must also be intact and the production nursery or facility must be in compliance with production requirements to maintain certification.
- All citrus production nursery stock and budwood moved from the certified growing areas must be accompanied by a state certificate documenting that the plant material meets state phytosanitary standards or the phytosanitary standards of the receiving state or country. This documentation must identify the citrus production nursery or budwood facility site.
- No plants under 6 months of age may be permitted outside of a quarantine zone, i.e. all plants must be grown for at least 6 months as this will give symptoms of HLB a strong chance of becoming evident.

8.3.2 Procedures for management of HLB (vector present)

Where HLB and a vector are present, use the below management strategies to contain the organisms. All of the above points also apply, although where contradictions occur, below points take precedents.

- Production of citrus nursery stock and budwood sources in approved structures. In Florida structures must be sealed and with gaps covered with a maximum screen size of 266 x 818 µm, designed to exclude psyllids, melon aphid and other aphids, leafminers or other pests. The structure should be subdivided with additional interior walls and doors to further preclude or minimize internal insect movement should insects be detected in one part of the structure. Any structure must include double entryways with positive pressure air displacement. If cooling pads and fans are used they must be enclosed with insect resistant screen as described above. Adequate construction materials should be kept available on site in the event that the structure is damaged so that timely repairs can be made. Any damages that are detected must be immediately reported to the appropriate official.
- An appropriate vector control prophylaxis program must be in place to facilitate control of potential exotic vectors in the structures. Also vector insecticide spray programs must be implemented in all outdoor production nurseries during the transition period (see section below).
- Use of sprayers (e.g. oscillating booms rather than airblast sprayers) that do not assist, or at least minimise, dispersal of adult psyllids in air currents.
- Geographical isolation of budwood sources (mother trees) from commercial citrus orchards; budwood facilities must be located at least 16 km away from commercial citrus production and away from concentrations of backyard citrus.
- Security and sanitation measures to prevent pest or disease introductions.
- Inspections to verify pest- and disease-freedom in citrus production nurseries and budwood facilities. Presence of significant pests and diseases could result in quarantine action for a period of time (if a significant pest or disease outbreak occurs within the structure then it could also be possible for HLB and its vectors to also be present) until the outbreak is controlled and certainty exists that HLB and its vectors are not present.
- Use of windbreaks to restrict and slow psyllid dispersal.
- Propagation of plants as inter-plants or ground-covers within orchards that produce volatiles that repel adult psyllids.
- Hedging and pruning practices timed and undertaken to minimise the risk of enhancing vector populations.

²⁰ Legislation will be required for mandatory removal of plants even in the absence of a positive HLB record (e.g., abandoned orchards).

²¹ Excluding rare and endangered native species of *Citrus*. Although these should be marked and surveyed for HLB symptoms.

- Strategies to encourage, where feasible, biological control of the vectors by their natural enemies (e.g., planting of groundcover plants to encourage generalist predators).
- Time irrigation (where feasible) and fertiliser applications to regulate the timing, number and extent of flush cycles; use of supplementary overhead irrigation to reduce psyllid populations.
- Monitor using yellow sticky traps placed about 1-2 m off the ground among relevant host plant species. This can help supplement more time consuming surveying, which should also be conducted, e.g. plant beating or sweep netting. In orchards, sticky traps should be placed in higher densities around the edges of the plot.

8.3.3 Chemical control

Management of citrus psyllids with pesticides is extremely important for their containment and eradication. Normally this requires a multiple applications per year, including systemic drenches and foliar sprays to reduce and maintain low vector populations. It is also important to treat trees with an appropriate pesticide prior to the removal (destruction) of a plant with HLB and its vectors to reduce spread of the vector (i.e. destroying a plant is likely to dislodge adults which are then likely to land and potentially infect another plant). Strategies to reduce psyllid populations using chemical control include:

- Strategically apply insecticides and mineral oils. Timing of sprays should be based on host-plant phenology so as to minimise feeding and oviposition and to maximise mortality of eggs, nymphs and adults. Applications of insecticides at critical flushing periods and during winter can greatly reduce populations of *D. citri* (Hall et al. 2013).
- Application of sprays should be even and thorough.
- Use of soil drenches and tree injections should be based on tree size and phenology and account for potential loss or diminution of active ingredient(s) through leaching, degradation or tree growth.
- Chemical rotation should be used to reduce the occurrence of insecticide resistance.
- Use a combination of longer-term soil-drenches or trunk injections with shorter term foliar sprays. Drenches require a number of weeks to be fully taken up by the plant and cause mortality to citrus psyllids, but when appropriately timed before flushing (e.g. late winter) can reduce populations over critical flushing periods.
- Current registrations for applications against citrus psyllids on citrus and nursery stock in Australia are limited to imidacloprid.
- Application for emergency minor use permits will be critical in the event of a detection of a citrus psyllid in Australia. Refer to appendix 9 for details on registrations in Australia and notes on use of chemicals overseas which may aid in this process.

Pesticide resistance has already been recorded overseas, with reduced efficacy associated with the use of certain neonicotinoid, organophosphate and pyrethroid products (Grafton-Cardwell et al. 2013). If resistant insect vectors establish in Australia, it could hamper our ability to successfully inhibit populations of citrus psyllids. Alternative products exist which are efficacious and these would have to be integrated into a robust pest management plan following successful application for emergency minor-use permits.

9 Course of action

Generic information on pest eradication is provided by the IPPC (1998b) in Guidelines for Pest Eradication Programmes. This standard describes the components of a pest eradication programme which can lead to the establishment or re-establishment of pest absence in an area. A pest eradication program may be developed as an emergency measure to prevent establishment and/or spread of a pest following its recent entry (re-establish a pest free area) or a measure to eliminate an established pest (establish a pest free area). The eradication process involves three main activities: surveillance (covered in section 7.2), containment and treatment and/or control measures (which has a high degree of overlap with management of HLB and citrus psyllids – section 7.3).

9.1 Destruction strategy

9.1.1 Destruction protocols

General protocols:

- No plant material should be removed from the quarantine area unless part of the disposal procedure or as part of regulated movement approved by biosecurity organisations, i.e. when plant material is grown in a pest free area, treated appropriately and is certified by a trained biosecurity inspector. If both HLB and its vectors are present the plants require pesticide treatment before destruction. At this time there are insufficient registrations or minor use permits to facilitate this process.
- Disposable equipment, infested plant material or growing media/soil should be disposed of by autoclaving, high temperature incineration or deep burial either on site or off-site (after containing the equipment in a sealed container that allows treatment of the container).
- Any equipment or plant material removed from the site for disposal should be double-bagged.
- Machinery (including vehicles that come onto the property) used in destruction processes need to be thoroughly washed, preferably using a detergent or farm degreaser.

9.1.2 Decontamination protocols

Machinery, equipment and vehicles in contact with infested plant material or growing media/soil, or present within the Quarantine Area, should be washed to remove plant material and growing media/soil. While HLB and its vectors are not found in growing media or soil, other pathogens may be present which could be spread by eradication efforts if they are not dealt with appropriately. High pressure water and agricultural detergent products, or physical scrubbing will generally remove most of the infective propagules. An appropriate disinfectant can then be applied after this, if required. Bleach solution (1% available chlorine) is a very effective disinfectant, although is corrosive on machinery and can be deactivated by detergents and organic matter. If alternative, effective disinfectants are available, they should be considered. Procedures of this nature should be undertaken using appropriate PPE (personal protective equipment). When using high pressure water, care should be taken not to spread plant material. High pressure water should be used in designated wash down areas which meet the following guidelines:

- Located away from crops or sensitive vegetation.
- Readily accessible with clear signage.
- Access to fresh water and power.
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting).
- Gently sloped to drain effluent away.
- Effluent must not enter water courses or water bodies.
- Allow adequate space to move larger vehicles.
- Away from hazards such as power lines.
- Waste water, growing media/soil or plant residues should be contained (see Appendix 18 of PLANTPLAN [Australia, 2010]).
- For inspection visits, hands, gloves, clothes, equipment and boots need to be clean on arrival and cleaned on departure. Clothes should be brushed down at the end of an inspection visit (double bag the brush, then freeze and disinfect). Vehicles need to be parked away from the infested area, and windows and doors need to be kept closed as much as possible.
- Ensure relevant safety equipment is used during clean up procedures, having consulted safety data sheets.

9.1.3 Priorities

- Stop the movement of any plant material that may be infested/infected with the pest/pathogen. If there is a high degree of certainty that an EPP is present a quarantine can be placed on the property immediately but this is unlikely to be possible for HLB, owing to the similarity of symptoms of native diseases and disorders. It is better to gain the cooperation of the property owner until the quarantine can be applied.
- Confirm the presence of the pest/pathogen.

- Limit movement of people and prevent movement of vehicles and equipment through affected areas.
- Determine the strategy for the eradication/decontamination of the pest and infested/infected host material.
- Determine the extent of infestation through survey and tracing information which would be assessed on a case by case basis and included within the response plan.

9.1.4 Trace-backs and trace-forwards

Trace-backs and forwards assist delimiting survey activities following an initial detection. Trace-backs attempt to determine the source of the infection whereas trace-forwards further define potential spread of and dissemination of the infection. There are many potential sources of trace backs/forwards. These are summarised to assist in the investigations to locate potential populations of HLB and its vectors. However, not all of these will be relevant to all scenarios so one must determine the importance of certain lines of investigation on a case by case basis.

Analysis of HLB symptoms may be useful for determining the age of the infection site and for conducting trace-back analysis. Symptoms on residential trees are not as predictable as commercial plants because of varying levels of horticultural care. The lag in time between transmission by psyllid vectors or propagation and the onset of symptoms can be quite variable and quantifying the severity of disease symptoms in individual trees may not be a true indication of pathogen concentration (Beattie and Barkley unpublished).

- Trace-backs
 - Investigate where the infected material may have been purchased, this may include:
 - Retail nursery, produce store, weekend or road-side market or internet sale
 - Production nursery – trace-back to mother stock plants
 - Motherstock plants (budwood) located off-farm
 - Self-propagated cutting.
 - Illegal importation
 - Via internet, private sale or human movement across Torres Strait
 - Legal importation
 - Investigate past or current infestations of citrus psyllids
 - Proximity to other host plants, citrus orchard, production nurseries etc. particularly if they are known to be infested with citrus psyllids
 - Investigate past activity of citrus psyllids (if not currently present) by examining damage and sooty mould on old growth
 - Storm or cyclone activity must be considered, particularly in northern Australia
 - Proximity to airports and major roads – hitchhikers
 - Proximity to juicing facilities (as citrus psyllids can be transported on fruit and or leaves destined for juicing).
- Trace-forwards
 - Local movement of psyllids to citrus or other host plants in residential and native environments
 - Long distance movement of plants via
 - Sale of plants
 - At production nurseries there should be records of where consignments of plants have been sold. Sales of all host plants of HLB and its vectors should be investigated from the last 6 months. Longer if deemed necessary (e.g. if the grower indicated that an importation occurred which may have been associated with the pest/pathogen in question and tracing each sale could result in having the EPP being declared as eradicated).
 - At retail outlets, markets etc will cause the scope of residential surveillance to be widened substantially
 - Within and around citrus orchards – particularly at edges

9.1.5 Disposal issues; plants, by-products and waste processing

- Any growing media/soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial. If both HLB and its vectors are

present, plant material must be inspected to insure that all citrus psyllids have been killed prior to transport off-site. This is best achieved using an insecticide application prior to removal of the plant. Ensure that it is safe to enter the site before doing so to avoid exposure to insecticides.

- Plant debris from the destruction zone must be carefully handled and transported.
- Infested areas or production nursery property should remain free of susceptible host plants until the area has been shown to be free from the pathogen and vector.
- Particular care must be taken to minimise the transfer of infected or infested plant material from the area.
- Host material should be collected and incinerated or double bagged and deep buried in an approved site. If possible, remove as much soil and leaf-litter as possible as these do not spread HLB or its vectors.
- The root system of in-ground host plants that have been destroyed should be poisoned to prevent regrowth which will be infected with HLB.

9.2 Containment strategies

For some exotic pest incursions where eradication is considered impractical, containment of the pest may be attempted to prevent or slow its spread and to limit its impact on other parts of the state or country. Containment is currently being considered for inclusion within the Emergency Plant Pest Response Deed (EPPRD). The decision on whether to eradicate or contain the pest will be made by the National Management Group, based on scientific and economic advice. Emergency interim containment measures are possible under EPPRD arrangements to gather information to determine if eradication is technically feasible. In any case, management actions suggested in section 8.3 will aid in the containment of HLB and its vectors.

9.3 Quarantine and movement controls

As per examples discussed in section 8.2.3, quarantines may be established at different radii prohibiting movement of all host plants species depending where the detection has occurred. Controlling the movement of plants infested by HLB and ACP is the first line of defense in preventing spread of these organisms to new areas. The approach taken here is that detections in isolated areas (without dense residential areas occurring within 5-15 km) have a lesser quarantine area than those in dense residential areas. In addition, areas with large numbers of host plants are more likely to produce large vector populations and therefore increase the size of the quarantine required. Thus, below we make recommendations as to the size quarantine areas under various scenarios. These scenarios are not exhaustive and should be treated as guidelines for situations not discussed here. Quarantines suggested here must be legislated in each state, as appropriate. Additional detections will increase the size of quarantine zones proportionally.

Guidelines for the size of quarantines under various scenarios

Description of detection area	Size of quarantine
Isolated areas, no citrus orchards, production nurseries or dense residential areas within 5 km (example 1)	5 km
Isolated areas at a citrus orchard, but no production nurseries or dense residential areas within 15 km (example 2)	15 km
Isolated areas at a production nursery with no dense residential areas within 10 km (example 3)	5 km*
In dense residential areas but no retail or production nurseries within 2km (example 4)	2 km
In dense residential areas and information is available to indicate that HLB and or its vectors have been distributed by retail outlets or are otherwise widely distributed (example 4)	Variable - consider if eradication is feasible and create a relevant sized quarantine zone based on information available.

* Modify as necessary based on trace-forward surveys.

The only method by which a business or entity may gain permission to send plant material which could be a host of HLB or its vectors is to follow management guidelines, as per section 8.3. Similar procedures have been established overseas and could be used as a basis for an official compliance document²². Vehicles, machinery and equipment (e.g. containers) associated with the production of host plants which can be host of HLB and its vectors require decontamination (as per section 8.1.2) prior to leaving the quarantine zone. There will also be an urgent need to identify, contact and prohibit internet sources from shipping host plants from/into quarantine and buffer areas and can be addressed by development of software to spider, download, index, and track the sale of these plants. Internet surveillance software will allow regulators to identify offending websites, contact the owners and track compliance (Rotstein et al. 2002).

9.4 Post-eradication surveillance

The period of pest freedom sufficient to indicate that eradication of the pest has been achieved will be determined by a number of factors, including growth conditions, the previous level of infection, the control measures applied and the pest biology.

Specific methods to confirm eradication of HLB and its vectors may include:

- Monitoring of sentinel plants
- Sentinel plants are to be grown in containers or small plots at the affected site. Plants are to be grown *in situ* under quarantine conditions and monitored for symptoms of infection
- If symptoms are detected, samples are to be collected and stored and plants destroyed
- Surveys comprising host plant sampling for the vector and the pathogen should be undertaken for a minimum of three years after eradication has been achieved
- Alternate non-host crops should be grown on the site and any self-sown plants sprayed out with a selective herbicide

10 Technical debrief and analysis for stand down

The response is considered to be ended when either:

- Eradication has been deemed successful by the lead agency, with agreement by the Consultative Committee on Emergency Plant Pests and the Domestic Quarantine and Market Access Working Group.
- Eradication has been deemed impractical and procedures for long-term management of the disease risk have been implemented.

A final report should be completed by the lead agency and the handling of the incident reviewed.

Eradication will be deemed impractical if, at any stage, the results of the delimiting surveys lead to a decision to move to containment/control.

11 References

²² See for example: <http://www.kernag.com/caap/detection/acp/acp-production-nursery-agreement-inside-quarantine-area.pdf>
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/CitrusSurveyProtocol-ExclusionaryFacilitiesInterstateMovement.pdf
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- <http://www.californiacitrusthreat.com>
- http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening
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- http://www.eppo.org/QUARANTINE/bacteria/Liberobacter_africanum/LIBESP_ds.pdf
- http://www.eppo.org/QUARANTINE/insects/Diaphorina_citri/DIAACI_ds.pdf
- http://www.eppo.org/QUARANTINE/insects/Trioza_erythrae/TRIZER_ds.pdf
- <http://ccpp.ucr.edu/news/PsyllidbrochureAug05.pdf>
- <http://www.padil.gov.au>
- <http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreening.html>
- <http://edis.ifas.ufl.edu/IN668>
- <http://entomology.ifas.ufl.edu/creatures/citrus/acpsyllid.htm>
- <http://anrcatalog.ucdavis.edu/pdf/8205.pdf>

http://cirs.ucr.edu/pdf/grafon-cardwell,beth-asian_citrus_psyllid_and_the_citrus_disease_huanglongbing.pdf
<http://www.ipm.ucdavis.edu/PMG/PESTNOTES/pn74155.html>
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/twg/cns-twg-report.pdf
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/downloads/chrp.pdf
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/CitrusSurveyProtocol-ExclusionaryFacilitiesInterstateMovement.pdf

13 Appendices

Appendix 1: Hosts of the Asiatic citrus psyllid ²³

Scientific name	Common name	Scientific name	Common name
<i>Aegle marmelos</i>	Bael	<i>Citrus wintersii</i>	Brown River finger lime
<i>Aeglopsis chevalieri</i>	Chevalier's aeglopsis	<i>Citrus x aurantiifolia</i>	West Indian, Key lime
<i>Afraegle gabonensis</i>	Gabon powder-flask-fruit	<i>Citrus x aurantium</i>	Oranges, grapefruit and tangelos
<i>Afraegle paniculata</i>	Nigerian powder-flask-fruit	<i>Citrus x junos</i>	Yuzu
<i>Atalantia buxifolia</i>	Chinese box orange	<i>Citrus x limon</i>	Lemons
<i>Atalantia monophylla</i>	Indian atalantia	<i>Citrus x microcarpa</i>	Calamandarin, calamondin, calamansi
<i>Balsamocitrus dawei</i>	Uganda powder-flask-fruit	<i>Citrus x taitensis</i>	Rough lemon
<i>Bergera koenigii</i>	Curry leaf	<i>Citrus x virgata</i>	Sydney hybrid
<i>Citropsis articulata</i>	West African cherry-orange	<i>Clausena anisumolens</i>	Kayumanis
<i>Citropsis gilletiana</i>	Gillet's cherry-orange	<i>Clausena excavata</i>	
<i>Citrus amblycarpa</i>	Nasranan	<i>Clausena indica</i>	
<i>Citrus australasica</i>	Australian finger-lime	<i>Clausena lansium</i>	Wampee, huangpi
<i>Citrus australis</i>	Australian round lime, dooja	<i>Limonia acidissima</i>	Indian wood apple, elephant apple
<i>Citrus cavaleriei</i>	Ichang (Yichang) papeda	<i>Merrillia caloxylon</i>	Kamuning, katinga, ketengah, Malay lemon
<i>Citrus glauca</i>	Australian desert lime	<i>Murraya paniculata, M. exotica</i>	Mock orange, orange jasmine, orange jessamine
<i>Citrus glauca</i> x Shakura		<i>Naringi crenulata</i>	Hesperethusa
<i>Citrus reticulata</i>		<i>Pamburus missionis</i>	
<i>Citrus hystrix</i>	Leech lime, limau purut, limau hantu, kaffir lime, Mauritius papeda	<i>Ravenia spectabilis</i>	
<i>Citrus inodora</i>	Russell River lime, large leaf Australian wild lime	<i>Swinglea glutinosa</i>	Tabog
<i>Citrus japonica</i>	Kumquat	<i>Tetradium ruticarpum</i>	Evodia, wu zhu yu

²³ Data based on Halbert and Manjunath (2004) and Beattie and Barkley (Unpublished)

Scientific name	Common name	Scientific name	Common name
<i>Citrus maxima</i>	Pomelo, pummelo	<i>Toddalia asiatica</i>	Orange-climber, forest pepper
<i>Citrus medica</i>	Citron	<i>Triphasia trifolia</i>	Limeberry, triphasia
<i>Citrus reticulata</i>	Mandarins	<i>Vepris lanceolata</i>	White ironwood
<i>Citrus trifoliata</i>	Trifoliolate orange	<i>Zanthoxylum fagara</i>	Lime prickly-ash

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Appendix 2: Hosts of the African citrus psyllid ²⁴

Scientific name	Common name	Scientific name	Common name
<i>Calodendrum capense</i>	Cape chestnut	<i>Citrus × limon</i>	Lemon
<i>Citrus australasica</i>	Australian finger-lime	<i>Clausena anisata</i>	Horsewood
<i>Citrus japonica</i>	Kumquat	<i>Murraya exotica</i>	Mock orange, orange jasmine, orange jessamine
<i>Citrus maxima</i>	Pomelo, pummelo	<i>Toddalia asiatica</i>	Orange-climber, forest pepper
<i>Citrus medica</i>	Citron	<i>Triphasia trifolia</i>	Limeberry, triphasia
<i>Citrus reticulata</i>	Mandarins and others	<i>Vepris</i> sp.	
<i>Citrus trifoliata</i>	Trifoliolate orange	<i>Vepris lanceolata</i>	White ironwood
<i>Citrus × aurantiifolia</i>	West Indian, Key lime	<i>Zanthoxylum capense</i>	Small knobwood
<i>Citrus × aurantium</i>	Oranges, grapefruit and tangelos		

²⁴ Data based on Beattie and Barkley (unpublished)

Appendix 3: Hosts of huanglongbing

Scientific name	Common name	Scientific name	Common name
<i>Aeglopsis chevalieri</i>	Chevalier's aeglopsis	<i>Citrus x junos</i>	Yuzu
<i>Atalantia buxifolia</i>	Chinese box orange	<i>Citrus x limon</i>	Lemons
<i>Balsamocitrus dawei</i>	Uganda powder-flask-fruit	<i>Citrus x latifolia</i>	Tahiti, Persian lime
<i>Bergera koenigii</i>	Curry leaf	<i>Citrus x microcarpa</i>	Calamandarin, calamondoin, calamansi
<i>Calodendrum capense</i>	Cape chestnut	<i>Citrus x taitensis</i>	Rough lemon
<i>Catharanthus roseus</i>	Periwinkle	<i>Clausena anisata</i>	False horsewood
<i>Citrus amblycarpa</i>	Nasranan	<i>Clausena indica</i>	
<i>Citrus australasica</i>	Australian finger- lime	<i>Clausena lansium</i>	Wampee, huangpi
<i>Citrus cavaleriei</i>	Ichang (Yichang) papeda	<i>Cuscuta</i> sp.	Dodder
<i>Citrus hystrix</i>	Leech lime, limau purut, limau hantu, kaffir lime, Mauritius papeda	<i>Cuscuta australis</i>	Dodder
<i>Citrus japonica</i>	Kumquat	<i>Limonia acidissima</i>	Indian wood apple, elephant apple
<i>Citrus maxima</i>	Pomelo	<i>Murraya exotica</i>	Mock orange, orange jasmine, orange jessamine
<i>Citrus medica</i>	Citron	<i>Nicotiana tabacum</i>	Tobacco
<i>Citrus reticulata</i>	Mandarins	<i>Pamburus missionis</i>	
<i>Citrus trifoliata</i>	Trifoliolate orange	<i>Solanum lycopersicum</i>	Tomato
<i>Citrus x aurantiifolia</i>	West Indian, Key lime	<i>Swinglea glutinosa</i>	Tabog
<i>Citrus x aurantium</i>	Oranges, grapefruit and tangelos	<i>Triphasia trifolia</i>	Limeberry
<i>Citrus x insitorum</i>	Troyer and Carrizo citrange	<i>Vepris lanceolata</i>	White ironwood

Appendix 4: Genera of native Australian plants in the family Rutaceae

<i>Acronychia</i>	<i>Euodia</i>	<i>Lunasia</i>	<i>Pitaviaster</i>
<i>Boronia</i>	<i>Flindersia</i>	<i>Luvunga</i>	<i>Sarcomelicope</i>
<i>Bosistoa</i>	<i>Geijera</i>	<i>Medicosma</i>	<i>Zanthoxylum</i>
<i>Citrus</i>	<i>Glycosmis</i>	<i>Melicope</i>	<i>Zieria</i>
<i>Clausena</i>	<i>Halfordia</i>	<i>Micromelum</i>	
<i>Dinosperma</i>	<i>Harrisonia</i>	<i>Murraya</i>	
<i>Eriostemon</i>	<i>Leionema</i>	<i>Phebalium</i>	

Appendix 5: Species of native citrus in Australia

Scientific name	Common name
<i>Citrus australis</i>	Australian round lime
<i>Citrus australasica</i>	Australian finger lime
<i>Citrus garrawayi</i>	Mount White lime
<i>Citrus glauca</i>	Australian desert lime
<i>Citrus gracilis</i>	Humpty Doo lime
<i>Citrus inodora</i>	Russell River lime

Appendix 6: Resources and facilities – diagnostic service facilities and psyllid diagnosticians in Australia

Facility	State	Details
DPI Victoria – Knoxfield Centre	Vic	621 Burwood Highway Knoxfield VIC 3684 Ph: 03 9210 9222; Fax: 03 9800 3521
DPI Victoria – Horsham Centre	Vic	Natimuk Rd Horsham VIC 3400 Ph: 03 5362 2111; Fax: 03 5362 2187
DPI New South Wales – Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: 02 4640 6327; Fax: 02 4640 6428
DPI New South Wales – Tamworth Agricultural Institute	NSW	4 Marsden Park Road Calala NSW 2340 Ph: 02 6763 1100; Fax: 02 6763 1222
DPI New South Wales – Wagga Wagga Agricultural Institute	NSW	PMB Wagga Wagga NSW 2650 Ph: 02 6938 1999; Fax: 02 6938 1809
SARDI Plant Research Centre – Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae SA 5064 Ph: 08 8303 9400; Fax: 08 8303 9403
Grow Help Australia	QLD	DAFF Ecosciences Precinct Dutton Park Q 4102 Ph: 07 3255 4365; Fax: 07 3846 2387
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: 08 9368 3721; Fax: 08 9474 2658
Department of Primary Industry and Fisheries	NT	Department of Primary Industry and Fisheries Plant Industries Division BAL building, Berrimah Farm, Makagon Road, Berrimah NT 0828 Ph: 08 8999 2261; Fax: 08 8999 2312
Psyllid diagnosticians in Australia		
Justin Bartlett*	Qld	Department of Agriculture, Fisheries and Forestry Biosecurity Queensland Ecosciences Precinct PO Box 267 Brisbane, Qld 4001 Ph: 07 3255 4357 Email: justin.bartlett@daff.qld.gov.au
Glenn Bellis*	NT	Northern Australia Quarantine Strategy (NAQS) PO Box 37846 Winnellie NT 0821 Ph: 08 8920 7024 Fax 08 8920 7033 Mob: 0417 271 324 Email: glenn.bellis@daff.gov.au
Peter Gillespie	NSW	Peter Gillespie Ph: 02 6391 3986 Email: peter.s.gillespie@dpi.nsw.gov.au
Lionel Hill*	Tas	Department of Primary Industries, Parks, Water & Environment P.O. Box 303 Devonport 7310, TASMANIA Phone: 03 6421 7636 Mob: 0418 379 726 Email: lionel.hill@dpiwve.tas.gov.au

Facility	State	Details
Mallik Malipatil	Vic	Department of Environment and Primary Industries Biosciences Research Division Associate Professor La Trobe University AgriBio, Centre for AgriBioscience 5 Ring Road Bundoora Vic 3083 Ph: 03 9032 7302; Mobile 0417359514 Email: mallik.malipatil@dpi.vic.gov.au
Andras Szito	WA	Plant Biosecurity Entomology Department of Agriculture and Food Western Australia Ph: 08 9368 3571 Fax: 08 9368 3808 Email: andras.szito@agric.wa.gov.au
Gary Taylor	SA	University of Adelaide Earth & Environmental Sciences The University of Adelaide, AUSTRALIA 5005 Ph: 08 8313 8347 Email gary.taylor@adelaide.edu.au

* Indicates that diagnostician is most familiar with *D. citri*, other psyllid species requiring identification may need to be sent to a different diagnostician.

Appendix 7: Communications strategy

A general Communications Strategy is provided in Appendix 6 of PLANTPLAN (Plant Health Australia, 2010).

Appendix 8: Market access impacts

Within the AQIS PHYTO database (www.aqis.gov.au/phyto) export of some material may require an additional declaration regarding freedom from huanglongbing. Latest information can be found within PHYTO, using an Advanced search “Search all text” for huanglongbing.

Appendix 9: Notes on insecticide registrations in Australia and overseas

Actives currently registered against other sucking pests on citrus in Australia

Active ingredient	Chemical group	Mode of action ⁱ	Pest
Aldicarb	1A	CSI	Soft brown scale, longtailed mealybug, others
Buprofezin	16	CV	Mealy bugs, scales and jassids
Carbaryl	1A	CIT	Various scales, spined citrus bug
Diazinon	1B	CIV	Spined citrus bug
Maldison	1B	CSI	Various scales and sucking bugs
Methidathion	1B	CI	Black citrus aphid and various scales
Methomyl	1A	CSI	Bronze orange and spined citrus bug, long tailed mealybug
Omethoate	1B	CSI	Aphids, bronze orange bug, mealybugs and red scale
Pyriproxyfen	7C	CT	Black scale and red scale
Spirotetramat	23	ITS	Various scales
Chlorpyrifos	1B	CIV	Scale
Various oils*	NA	C	scales, aphids

ⁱ Abbreviations stand for: C = contact, S = systemic, I = ingestion, V = volatile, T = translaminar.

* Likely to only be valuable in addition to another product

Products registered for psyllids in other crops in Australia:

Active ingredient	Chemical group	Mode of action	Pest
Acetamiprid	4A	CIS	Lilly pilly
Clothianidin	4A	CIS	Eucalyptus and sandalwood
Imidacloprid	4A	CIS	Citrus, ornamentals and many others
Methidathion	1B	CI	Forest timber production, gardens, ornamentals, ornamental trees, shrubs
Methomyl	1A	CSI	Melaleucas (teatrees) - oil

Overseas

- Spirotetramat is registered against potato psyllids in NZ and is also registered against psyllids in the US
- Pyriproxyfen is active against the Asiatic citrus psyllid:
<http://www.crec.ifas.ufl.edu/academics/faculty/stelinski/PDF/Boinaetal.PMS2010.pdf> and is registered for some psyllids in the USA
- Spinosad is used against psyllids in the USA and is registered in Australia against fruit fly, caterpillars and thrips on ornamentals and ornamental trees (Group 5).

- Bifenthrin has been shown to be effective (<http://www.fshs.org/Proceedings/Password%20Protected/2006%20v.%20119/FSHS%20119/p.417-421.pdf>) and is registered against aphids in Australia, and a few other sucking insects on ornamentals and against citrus leaf eating weevil on citrus.
- Buprofezin is also effective against *D. citri* but is most effective against young insects. <http://www.ncbi.nlm.nih.gov/pubmed/22653617>

Appendix 10: Important nursery and garden industry contacts

It is important to note that the Industry Development Officers (IDOs) change from time to time. Therefore, the current list may become out of date relatively quickly. For this reason, one can always refer to the NGIA website for the latest details for the NGI for each state and territory. In addition, some states may have more than one IDO, the below list are important contacts who may then direct you to the most appropriate person.

Northern Territory

Website:

http://www.ngia.com.au/Category;jsessionid=57FAB91F4A656937462E3F34A910B531?Action=View&Category_id=266

Michele Shugg
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Nursery & Garden Industry Northern Territory
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Email: ngint@ntha.com.au

Megan Connelly
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GPO Box 3000 Darwin, NT 0801
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NSW and ACT

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Michael Danelon
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Fax: (02) 9679 1655
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Email: michael@ngina.com.au

Queensland

Website: www.ngiq.asn.au

John McDonald
Industry Development Manager
Telephone: 07 3277 7900
Mobile: 0419 683 457
Fax: +61 (07) 3277 7109
Email: nido@ngiq.asn.au

South Australia

Website: www.ngisa.com.au

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505 Fullarton Rd (Gate A)
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