

CITRUS INDUSTRY BIOSECURITY PLAN

Pest Risk Review

COMMON NAME:	Huanglongbing (Citrus greening)
SCIENTIFIC NAME:	<i>Candidatus Liberobacter asiaticus</i> Garnier [Proteobacteria]
SYNONYMS:	<i>Liberobacter asiaticus</i>

The scientific and technical content of this document is current to the date published and all efforts were made to obtain relevant and published information on the threat. New information will be included as it comes to light, or when the document is reviewed.

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BACKGROUND

Huanglongbing (HLB), also known as citrus greening, is a bacterial disease. Symptoms of HLB include yellowing of leaves, dieback, and distorted and discoloured fruit. Originally it was thought that cases of HLB observed in Asia and in Africa were caused by the same pathogen, however it has since been demonstrated that two different species of bacteria are responsible. In Asia HLB is caused by the phloem-limited bacterium *Candidatus Liberibacter asiaticus*, whilst in Africa *Candidatus Liberibacter africanus* is the causal agent. HLB is the most serious impediment to citriculture in Asia and is capable of destroying orchards within five years of planting (Beattie, 2002). Both pathogens are graft-transmissible, however psyllid vectors play a large role in spread of the disease. Chemical control of the vector of *C. L. asiaticus*, particularly in Thailand, has been of limited efficacy, with 35-52 sprays applied each year in some orchards since 1980 (Beattie, 2002).

PART OF PLANT/COMMODITY AFFECTED

Leaves, stems, flowers, fruit and roots. All growth stages of the plant are affected (e.g. seedling stage, vegetative growing stage, flowering stage, and fruiting stage). (CAB International, 2002).

BIOLOGY

Symptoms

The first visible sign of HLB is usually a yellow shoot in the canopy. Progressive yellowing of the rest of the canopy follows. Affected leaves are blotchily mottled, become pale yellow, or have the appearance of foliage affected by zinc and other nutrient deficiencies. These symptoms appear together on branches. New leaves are typically thin, yellow and upright. Leaves senesce early. The most characteristic symptom is blotchy mottle; however this symptom is not specific to HLB. The mottling reduces over time, leaving the deficiency symptoms most prominent. (CAB International, 2002).

Other circumstances causing a similar blotchy mottle pattern include water logging, use of marcots, or infection with stubborn disease (*Spiroplasma citri*), citrus tristeza closterovirus (CTV) or species of *Phytophthora*. Citrus blight also causes symptoms of zinc deficiency, however HLB affected trees do not display the wilting characteristic of blight. (CAB International, 2002).

Chronically infected trees exhibit extensive twig dieback and are sparsely foliated. Inflorescences ripen prematurely. HLB causes fruit symptoms similar to those caused by CTV. Fruits or pods are often small, contain aborted seeds, show distortion in shape, are poorly coloured and/or are abnormally patterned. Premature fruit drop can also occur. (CAB International, 2002).

Identification

Symptoms of HLB may be confused with stubborn disease, CTV infection, *Phytophthora* infection, citrus blight or certain nutrient deficiencies. This makes HLB difficult to identify in the field, however a yellowing canopy, blotchy mottled leaves and small lopsided fruits with aborted seeds are a good indication. Diagnosis must then be confirmed through use of EM, DNA hybridisation or PCR to identify the bacterium. The HLB bacteria are restricted to phloem sieve tubes in affected plants. They have also been observed in the haemolymph and salivary glands of the insect vectors. (CAB International, 2002).

Life history

Candidatus L. asiaticus has one known insect vector. This is the Asiatic citrus psyllid (*Diaphorina citri* Kuwayama [Hemiptera: Psyllidae]). The African citrus psyllid (*Trioza erytreae*) is the only known vector of the related pathogen *Candidatus L. africanus*. Each of these has proven capable of hosting either pathogen in the laboratory; however it is not known whether this occurs in nature. Multiplication of the pathogen does occur within the vector however no transovarial transmission occurs. (CAB International, 2002).

D. citri can acquire *C. L. asiaticus* from a 15 to 30 minute feeding period on an infected host plant. *D. citri* then retains the pathogen for life. An incubation period of approximately 21 days is required before *D. citri* is able to transmit the pathogen to other plants during feeding. Fourth or fifth instar *D. citri* nymphs and adults are capable of HLB transmission. (CAB International, 2002).

C. L. asiaticus and *C. L. africanus* differ in heat tolerance. Symptoms are not displayed above 25-30°C for the African species, whereas the Asian pathogen is more tolerant to high temperatures (symptoms displayed above 30°C). In South Africa, Kenya, Ethiopia, Madagascar and Yemen HLB (caused by *C. L. africanus*) is only found in cool climatic conditions and at elevations over 600-1000m. These conditions are unlikely to occur in the Australian citrus growing regions. In the Indian Subcontinent, Asia and Saudi Arabia, where *C. L. asiaticus* is the causal agent, the disease also occurs at low elevations with a hot climate. (CAB International, 2002).

Dispersal

HLB can be transferred via movement of infected tissue including bark, fruits, inflorescences, leaves, seedlings, micropropagated plants, roots, stems and wood. The pathogen can also survive and be transported between sites in growing media. (CAB International, 2002).

In the field both pathogens can be transmitted by dodder (*Cuscuta campestris*) from affected citrus trees to the alternative host *Catharanthus roseus*. The liberobacters reach higher titres in this host than in citrus. Transmission from *C. roseus* to *C. roseus* occurs via graft inoculation. Transfer of *C.L. asiaticus* from *C. roseus* to tobacco (*Nicotiana tabacum* var. *xanthi* NC) has also been demonstrated.

Severe symptoms are displayed by infected tobacco plants even though the liberobacter titre is relatively low. (CAB International, 2002).

Spread also occurs via movement of insect vectors. As *C. L. asiaticus* and *D. citri* have spread through eastern Indonesia there is a high risk that they could spread to Australia, either by natural means or by unintentional human-assisted introduction on host material (Beattie, 2002).

HOST RANGE AND DISTRIBUTION

HLB affects most commercial citrus types, particularly sweet oranges, tangelos and mandarins. Limes, pomelos and trifoliate oranges are less strongly affected. A range of other rutaceous plants including ornamentals and wild species are susceptible to infection; however the severity of symptoms displayed varies. In South Africa *Candidatus L. africanus* has been found in *Toddalia lanceolata*, a wild rutaceous species, and in *Calodendrum capense*, an ornamental rutaceous tree. In Asia, *Murraya paniculata*, an ornamental rutaceous shrub, is a preferred host of *D. citri*. (CAB International, 2002).

Primary host range

Rutaceae: *Citrus*, *Citrus sinensis* (navel orange), *Citrus reticulata* (mandarin), *Citrus reticulata* x *paradisi* (tangelo). Cuscutaceae: *Cuscuta campestris* (dodder), *C. reflexa* (giant dodder). (NB: These are hosts of HLB. *C. L. asiaticus* and *C. L. africanus* are not differentiated here). (CAB International, 2002).

Other hosts

Rutaceae: *Citrus aurantiifolia* (lime), *Poncirus*, *Fortunella*, *Murraya*, *Toddalia*, *Calodendrum capense*, *Severinia buxifolia*, *Limonia acidissima*. Apocynaceae: *Catharanthus roseus* (Madagascar periwinkle). (NB: These are hosts of HLB. *C. L. asiaticus* and *C. L. africanus* are not differentiated here).

Distribution

HLB caused by *C. L. asiaticus* is found in many countries throughout Asia, and is spreading in Indonesia. *C. L. africanus* occurs in numerous countries in Africa and is also spreading. HLB and its vectors have not been detected in Australia.

The following countries are listed as having HLB by CAB International (2002); however the two pathogen species are not differentiated.

List of countries

Asia

- Bangladesh: present, no further details (Catling et al., 1978)
- Cambodia: present, no further details (Garnier & Bové, 1998)

- China
 - o Fujian: present, no further details (Lin & Lin, 1956)
 - o Guangdong: present, no further details (Lin & Lin, 1956)
 - o Guangxi: present, no further details (Lin & Lin, 1956)
 - o Hainan: present, no further details (Lin & Lin, 1956)
 - o Jiangxi: present, no further details (Lin & Lin, 1956)
 - o Taiwan: widespread (Matsumoto et al., 1961)
 - o Zhejiang: present, no further details (Lin & Lin, 1956)
- India
 - o Andhra Pradesh: present, no further details (Bové et al., 1993)
 - o Assam: present, no further details (Ahlawat, 1997)
 - o Delhi: present, no further details (Bové et al., 1993)
 - o Indian Punjab: present, no further details (Ahlawat, 1997)
 - o Karnataka: present, no further details (Bové et al., 1993)
 - o Maharashtra: present, no further details (Bové et al., 1993)
 - o Orissa: present, no further details (Bové et al., 1993)
 - o Rajasthan: present, no further details (Bové et al., 1993)
 - o Uttar Pradesh: present, no further details (Bové et al., 1993)
- Indonesia
 - o Java: present, no further details (Aubert et al., 1985)
 - o Kalimantan: present, no further details (Aubert et al., 1985)
 - o Lesser Sunda Islands: widespread (Bové et al., 1998)
 - o Sulawesi: present, no further details (Aubert et al., 1985)
 - o Sumatra: present, no further details (Aubert et al., 1985)
- Japan
 - o Ryukyu Archipelago: present, no further details (Miyakawa & Tsuno, 1989)
- Laos: present, no further details (Garnier & Bové, 1998)
- Malaysia
 - o Peninsular Malaysia: present, no further details (Garnier & Bové, 1996)
 - o Sarawak: present, no further details (Bové et al., 1993)
- Myanmar: present, no further details (Garnier & Bové, 1998)
- Nepal: widespread (Regmi et al., 1996)
- Pakistan: present, no further details (Garnier & Bové, 1996)
- Philippines: widespread (Garnier & Bové, 1996)
- Saudi Arabia: restricted distribution (Bové & Garnier, 1984)
- Thailand: present, no further details (Promintara, 1990)
- Vietnam: restricted distribution (Bové et al., 1996)
- Yemen: restricted distribution (Bové & Garnier, 1984)

Africa

- Burundi: present, no further details (Aubert et al., 1988)
- Cameroon: present, no further details (Aubert et al., 1988)
- Central African Republic: present, no further details (Aubert et al., 1988)
- Ethiopia: present, no further details (Aubert et al., 1988)
- Kenya: present, no further details (Garnier & Bové, 1996)
- Madagascar: present, no further details (Bové & Garnier, 1994)
- Malawi: present, no further details (Aubert et al., 1988)

- Mauritius: present, no further details (Garnier et al., 1996)
- Rwanda: present, no further details (Aubert et al., 1988)
- Réunion: present, no further details (Etienne & Aubert, 1980)
- Somalia: present, no further details (Bové, 1995)
- South Africa: restricted distribution (Korsten et al., 1996)
- Swaziland: present, no further details (Bové & Garnier, 1994)
- Tanzania: restricted distribution (Bové & Garnier, 1994)
- Zimbabwe: restricted distribution (Garnier & Bové, 1996)

Potential distribution in Australia

CSIRO CLIMEX model predictions indicate that Asiatic citrus psyllid would survive in more than 50 per cent of the area covered by Australia's citrus-growing regions (Beattie, 2002).

The following risk analysis for HLB is based on the methodology in Biosecurity Australia's guidelines on Import Risk Analysis for Plants and Plant Products (2001).

ENTRY POTENTIAL

Entry potential: Rating = High

There is a high risk of *C. L. asiaticus* and *D. citri* being introduced, particularly by natural or unintentional human-assisted spread through Indonesia and Papua New Guinea, on either known or unknown hosts.

ESTABLISHMENT POTENTIAL

Establishment potential: Rating = High

CSIRO CLIMEX model predictions indicate that Asiatic citrus psyllid would survive in at least half of Australia's citrus-growing regions (Beattie, 2002).

SPREAD POTENTIAL FOLLOWING ESTABLISHMENT

Spread potential following establishment: Rating = High

For *C. L. asiaticus* with its psyllid vector.

ECONOMIC IMPACT

Economic impact: Rating = High

The trends in south east Asia indicate that in the presence of the insect vector, and without effective control, *C. L. asiaticus* would cause a significant and potentially unrecoverable decline in the Australian citrus industry over 10 to 20 years. The effects of the disease on productivity are often severe, particularly for sweet oranges, mandarins and tangelos.

With effective control, increased production costs may still significantly reduce the viability of the industry. Impacts on the industry are likely to be far greater than the potential loss of export income. (Beattie, 2002).

ENVIRONMENTAL IMPACT

Environmental impact: Rating = Unavailable (pending further research)

This disease has the potential to infect native rutaceous species.

CONCLUSIONS

Overall risk: Rating = Unavailable (pending finalisation of environmental impact rating)

REFERENCES

Beattie, A. (2002). Huanglongbing and Asiatic citrus psyllid in Asia and Australasia: overview, research objectives and incursion management. *Background paper: Citrus Industry Biosecurity Planning Workshop, Telopea Park Inn, Canberra, 25-26 November 2002*. Department of Agriculture, Fisheries and Forestry – Australia, Canberra.

Biosecurity Australia. (2001) *Guidelines for Import Risk Analysis: Draft September 2001*. Department of Agriculture, Fisheries and Forestry – Australia, Barton, ACT.

CAB International (2002). *Crop Protection Compendium, Global Module, 4th edition*. CAB International, Wallingford, UK.

The following references relate to the CAB International (2002) HLB geographic distribution list:

Ahlawat, Y.S. (1997). Virus, greening bacterium and viroids associated with citrus (Citrus species) decline in India. *Indian Journal of Agricultural Sciences*, **67**:51-57.

Aubert, B., Garnier, M., Guillaumin, D., Herbagyandono, B., Setiobudi, L. and Nurhadi, F. (1985). *Fruits*, **40**(9):549-563.

Aubert, B., Garnier, M., Cassin, J. C. and Bertin, Y. (1988). Citrus greening survey in East and West African countries south of the Sahara. Pages 231-237 in: (Garnsey, S. M., Timmer, L. W. and Dodds, J. A. eds). *Proceedings of the 10th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Bové, J. M. (1995). Pages 283-302 in: *Virus and virus-like diseases of citrus in the Near East region*. FAO, Rome, Italy.

Bové, J. M. and Garnier, M. (1984) Citrus greening and psylla vectors of the disease in the Arabian Peninsula. Pages 109-114 in: (Garnsey, S. M., Trimmer, L. W. and Dodds, J. A. eds). *Proceedings of the 9th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Bové, J. M. and Garnier, M. (1994). Citrus greening disease and its bacterial agent. *Proceedings of the International Society for Citriculture*, **3**:1283-1289.

Bové, J. M., Garnier, M., Ahlawat, Y. S., Chakraborty, N. K. and Varma, A. (1993). Detection of the Asian strains of the greening BLO by DNA-DNA hybridization in indian orchard trees and Malaysian *Diaphorina citri* psyllids. Pages 258-263 in: (Moreno, P., da Graça, J. V. and Timmer, L. W. eds). *Proceedings of the 12th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Bové, J. M., Chau, N. M., Trung, H. M. Bourdeaut, J. and Garnier, M. (1996). Greening in Vietnam. Pages 258-266 in: *Proceedings of the 13th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Bové, J. M., Erti Dwiastuti, M., Tiwiratno, A., Supriyanto, A., Nasli, E., Becu, P. and Garnier, M. (1998). In: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 14th Conference of the International Organization of Citrus Virologists*, in press.

Catling, H. D., Garnier, M. and Bove, J. M. (1978). Presence of citrus greening disease in Bangladesh and a new method for rapid diagnosis. *FAO Plant Protection Bulletin*, **26**(1):16-18.

Etienne, J. and Aubert, B. (1980). Biological control of psyllid vectors of greening disease on Reunion island. Pages 118-121 in: (Calavan, E. C., Garnsey, S. M. and Timmer, L. W., eds). *Proceedings of the 8th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Garnier, M. and Bové, J. M. (1996). Distribution of the Huanglongbing (greening) *Liberobacter* species in fifteen African and Asian countries. Pages 388-391 in: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 13th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Garnier, M. and Bové, J. M. (1998). Huanglongbing in Cambodia, Laos and Myanmar. In: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 14th Conference of the International Organization of Citrus Virologists*, in press.

Garnier, M., Jagoueix, S., Toorawa, P., Grisoni, M., Mallessard, R., Dookun, A., Saumtally, S., Autrey, J. C. and Bové, J. M. (1996). Both Huanglongbing (greening) *Liberobacter* species are present in Mauritius and Reunion. Pages 392-394 in: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 13th*

Conference of the International Organization of Citrus Virologists. IOCV University of California, Riverside, USA.

Korsten, L., Jagoueix, S., Bové, J. M. and Garnier, M. (1996). Huanglongbing (greening) detection in South Africa. Pages 395-398 in: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 13th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Lin, K.H. and Lin, K.H. (1956) The citrus huanglongbing disease in China. *Acta Phytopathologica Sinica*, **2**:14-38.

Matsumoto, T., Wang, M. C. and Su H. J. (1961). Studies on likubin. Pages 121-125 in: (Price, W. C. ed). *Proceedings of the 2nd Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.

Miyakawa, T. and Tsuno, K. (1989). Occurrence of citrus greening disease in the southern islands of Japan. *Annals of the Phytopathological Society of Japan*, **55**(5):667-670.

Promintara, M. (1990). Simultaneous infection of mandarin (*Citrus reticulata* Bl.) with tristeza virus and greening organism in Thailand. Pages 96-99 in: (Aubert, B., Tontyaporn, S. and Buangsuwon, D., eds). *Proceedings of the 4th International Asia Pacific Conference on Citrus Rehabilitation, Chiang Mai, Thailand, 4-10th February 1990*. FAO-UNDP RAS/86/022 Regional Project.

Regmi, C., Garnier, M. and Bové, J. M. (1996). Huanglongbing (greening) in Nepal. Pages 267-270 in: (Moreno, P., da Graça, J. V. and Timmer, L. W., eds). *Proceedings of the 13th Conference of the International Organization of Citrus Virologists*. IOCV University of California, Riverside, USA.