REPORT ON
INTERNATIONAL MASTER CLASS

Surveillance, Identification and Management of Citrus Diseases – Huanglongbing (HLB)

JAVA, INDONESIA
22ND JANUARY – 4TH FEBRUARY 2011

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Australian Government
Australian Centre for International Agricultural Research

THE CRAWFORD FUND
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Introduction

Huanglongbing (HLB) is the most severe impediment to citriculture in Asia and, as a consequence, seriously affects the welfare of farmers. What was a problem restricted largely to Asia before 2000 is now a world problem that threatens the very viability of commercial citrus production, a major world source of vitamin C. What was a problem for generally poor small-scale farmers in Asia is now a problem for wealthier, larger-scale commercial producers throughout the world.

The disease is caused by phloem-limited, Gram-negative liberibacters (α-Proteobacteria): a heat-tolerant form '*Candidatus* Liberibacter asiaticus', two heat-sensitive forms, '*Ca*. L. africanus' and '*Ca*. L. americanus', and a heat sensitive subspecies, '*Ca*. L. africanus ssp. capensis' (Bové 2006). In Asia, New Guinea and the Americas the disease is transmitted by the Asiatic citrus psyllid, *Diaphorina citri.* In Africa, it is transmitted by the African citrus psyllid, *Trioza erytreae*. In parts of the Arabian Peninsula and in Mauritius and Réunion, it may be transmitted by either or both psyllids.

'*Ca.* L. asiaticus' is the most widespread form. It occurs where *D. citri* occurs in Asia, Indian Ocean Islands, through the Indonesian Archipelago to New Guinea. It is spreading rapidly in the Americas and can destroy orchards within 5 years of planting: 100% infection of initially pathogen-free trees can occur within 2 years of planting (Yang *et al*. 2006). '*Ca*. L. americanus' has only been recorded in Brazil (Bové 2006, Lopes *et al*. 2010). '*Ca*. L. africanus' occurs in sub-Saharan Africa, Arabia and the Indian Ocean Islands of Mauritius and Réunion (Bové 2006). '*Ca*. L. africanus ssp. capensis' is only known to occur in southern Africa (da Graça 1991, Bové 2006). Use of insecticides does not prevent spread of the pathogens by the vectors.

The disease and its vectors are not present in Australia (Bellis *et al*. 2005, Beattie & Barkley 2009), but both '*Ca*. L. asiaticus' and *D. citri* now occur in New Guinea (Davis *et al*. 2005). Inevitable movement of the disease and *D. citri* eastward from Asia, to and through Australasia, will represent a serious threat to the Australian citrus industry, and to the region's biodiversity through the loss of citrus species and relatives that are endemic to Australasia. Some 50% of about 25 true species of Citrus are endemic to the region.

Through projects funded by the Australian Centre for International Agricultural Research (ACIAR), Horticulture Australia Limited (HAL), and the Department of Innovation, Industry, Science and Research (DIISR), and collaboration with scientists in Asia, Australian scientists, particularly Prof Andrew Beattie and A/Prof Paul Holford at the University of Western Sydney, and Pat Barkley, Citrus Australia, have broad international expertise with the disease and the Asiatic citrus psyllid. Andrew Beattie and Pat Barkley recently prepared an incursion management plan for Australia (Beattie & Barkley 2009).

During an ACIAR HLB workshop in Sydney in November 2009, it was noted that extensive research supported by ACIAR, and the importance of HLB overseas and in Australia, made surveillance, identification and management of the disease an eminently suitable topic for a Crawford Fund Master Class.

The overarching objective of the Master Class was to increase regional expertise, networking and collaboration among scientists working in Asia and Australasia on huanglongbing in order to reduce its impact on commercial citrus production and threatened germplasm.

Objectives

To:

- Enhance participants' skills in HLB surveillance, identification and management;
- Develop strategies, procedures and policy requirements for implementing viable, sustainable and effective local or area-wide mitigation practices for HLB and other pathogens in situations ranging from small to large farms in regions with low to high potential for disease ingress;
- Develop an online diagnostic manual for HLB infections in regionally grown citrus species, varieties, and alternative hosts;
- Where possible, determine new host records for liberibacters and phytoplasmas in plants and in *D. citri* and other possible vectors; and
- Examine the symptomatology of single and mixed infections of liberibacters, phytoplasmas and *Citrus* tristeza virus in citrus.

Administrative Organisation

This Master Class program was designed and administered by Prof G. Andrew C. Beattie and A/Prof Paul Holford, University of Western Sydney (UWS), Australia and Prof Siti Subandiyah, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. The organisers would like to thank staff from the Research Institute for Citrus and Subtropical Horticulture, Tlekung, Indonesia for hosting the presentation sessions held at the institute and for organising visits to farmers in the Batu region. The organisers would also like to thank staff and students from UGM for their help throughout the program.

Master Class Program

The program consisted of the following elements:

- Presentations given by the participants on the current status of HLB and the symptoms commonly seen on species and varieties of *Citrus* in their countries;
- Presentations given by the organisers on aspects of HLB research;
- Visits to farmers' fields and nurseries to observe the vector of HLB, to see symptoms of HLB and other *Citrus* diseases and to collect samples for laboratory analysis;
- Laboratory sessions providing training in:
    - the detection of CTV by direct tissue blotting and by reverse transcription PCR
    - the detection of HLB and phytoplasmas by conventional PCR and of HLB by real-time PCR
    - techniques for the preparation and observation of tissues samples by microscopy; and
- Discussions on strategies for controlling HLB.

The day-to-day Master Class program is presented in Appendix 1.

Master Class Participants

In total, 46 participants took part in the workshop either as organisers, resource personnel or participants of which 23 were female and were 23 male. The participants were from 16 countries: Australia (13); Bhutan (1); Brazil (1); Cambodia (1); China (2); Fiji (1); India (1); Indonesia (10); Laos (1); Malaysia (2); Pakistan (3); Thailand (2); Timor Leste (3); Tonga (1); USA (1) and Viet Nam (2).

The organisers would like to thank Marcos da Cruz (Secretary of State for Agriculture and Arboriculture, Timor Leste) and Gil Rangel da Cruz (National Director for Agriculture and Horticulture, Timor Leste) for their participation in the final few days of the workshop.

The names and contact details of all participants are presented in Appendix 2

Resource Personnel

The presentations were hosted by the organisers of the workshop. Dr Susan Halbert (Florida Department of Agriculture and Consumer Services, USA), Dr Silvio Lopes (Fundo de Defensa da Citricultura, Brazil), Mr Richard Davis (Northern Australia Quarantine Strategy, Australia), Dr Lily Eng (Agriculture Research Centre, Sarawak, Malaysia), Dr Joanne Slattery (Plant Health Australia), Dr Ratana Sdoodee (Prince of Songkla University, Thailand) and Dr Farman Ullah (Khyber Pakhtunkhwa Agricultural University, Pakistan) led field exercises. Lynne Jones (Northern Australia Quarantine Strategy, Australia), Grant Chambers (Industry and Investment NSW, Australia) and Elizabeth Kabanoff (UWS, Australia) together with the organisers designed and ran the practical sessions held at UGM. Eric Craswell represented the Crawford Fund at opening ceremonies in Malang and Yogyakarta.

Presentation of Master Class Certificates

Master Class certificates were presented at the closing session by Ms Frances Barns, Country Manager (Indonesia), Australian Centre for International Agricultural Research (ACIAR). An example of the certificate is given in Appendix 3.

Publications

Two media releases were made in Indonesia: "*Huanglongbing masih jadi momok tanaman jeruk*" on 23rd February 2011 and "*Threat by deadly citrus pest being addressed*" on 2nd March 2011. Extensive coverage of a Crawford Fund press release in Australia is shown in Appendix 4, together with the press release itself.

A DVD and publication are in preparation. The DVD will contain the presentations given by participants, photographs taken during the Master Class and literature on huanglongbing and it its vector. The publication will contain annotated symptoms of HLB commonly expressed on species and varieties in the participants' countries of origin. Specimens collected during the workshop are being analysed in the laboratories at UWS.

Lessons Learned

1. The biosecurity implications for Australia of an HLB incursion are so important that this Master Class attracted an unusually high number of self-funded participants from Australia. Furthermore, the support given to participants from HLB-affected regions of Brazil and the United States of America added to the numbers participating in the Master Class. The result was a higher total number and a much high proportion of participants from industrialised countries than the 15–20 participants normally expected in a Crawford Fund Master Class. This benefited the Master Class as it allowed participants with different backgrounds with HLB and other aspects of plant protection to work together and share knowledge and experiences. Moreover, it allowed those with greatest experience to train those with less experience. This synergy was enhanced by organising the participants into small groups, which worked together to collect and test samples in the field and processing these samples in the laboratory.

2. This Master Class was hosted by a university—UGM—whereas Master Classes commonly involve, or are hosted by, one of the international agricultural research centres. The success of the Master Class owes much to the excellent organisational and management skills of UGM staff, and the availability in Yogyakarta and Malang of hotel accommodation that was both comfortable and reasonably priced.

3. The participation of the Secretary of State for Agriculture and Arboriculture and the National Director of Agriculture and Horticulture from East Timor in the last few days of the Master Class created a better awareness at the highest level in the government of that country of the risks that HLB presents to citrus production.

4. The 28 responses to the post-Master Class survey indicate a very positive benefit to the skills and knowledge of individuals (see below). Most comments are positive, particularly in regard to the organisation of the Master Class. Comments also stress how much their awareness of HLB and its impact in the Asia-Pacific region and globally has increased as a result of their participation.

Summary of Feedback from Participants

The feedback questionnaire was completed by 28 of the participants. The results of the likert questions are given in the tables below and typical answers to the open questions are provided.

A As a result of the Master Class I:

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	Not Relevant
(1) Am able to apply the skills/knowledge from the training to my work	18	9	1	0	0	0
(2) Will continue to use the skills/knowledge learnt in the training in my current employment	17	10	1	0	0	0
(3) Work more effectively and efficiently	14	7	6	0	0	0
(4) Increased my competency and confidence in my work	12	14	1	0	0	0
(5) Increased my professional collaboration with people both nationally and internationally	19	8	1	0	0	0
(6) Increased my ability to continue to research in my subject area	11	14	1	0	0	0
(7) Was able to secure resources for further research	7	10	10	0	0	1
(8) The knowledge gained from the course enables me to influence government policy	3	12	11	2	0	0
(9) The networks made during the training will enable me to produce better research outputs	11	12	4	0	0	0
(10) The networks made during the training will enable me to produce better policy outputs	9	10	9	0	0	0
Average	12.1	10.6	4.5	0.2	0	0.1

(11) Other:

"Myself + probably plenty of others also benefitted greatly from opportunities to see firsthand other important diseases, pest and weeds not present at home"

"I am more motivated to find a long term solution to the problem"

"Stronger position in dealing with government policy"

B Personal - What sort of impact may the Crawford Fund training have on your current work situation? Please rate the statements bellow

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
(1) The training has no impact on my work situation	1	4	2	8	13
(2) The training will enable me to perform better at work	11	15	1	1	0
(3) The training may enable me to move to another position in my workplace	1	2	16	6	3
(4) I have more opportunities to collaborate with international and national organisations	13	15	0	0	0
(5) I will look at pursuing research further in my field	8	16	3	1	0
(6) I will look at other work opportunities in the field of the training	3	11	10	4	0
(7) I will look at further training opportunities	8	15	5	0	0
Average	6.4	11.1	5.3	2.9	2.3

(8) We would welcome any further comments you have about the impact the training activity may have on your life: (What was the most significant impact of the training for you personally?)

"As a result of feedback discussions from people doing fieldwork on HLB I will be altering/improving field survey methodology"

"*I am just very happy to be part of this training and to interact with many participants from different countries with diverse backgrounds on entomology and pathology. The greatest impact is definitely that I will be able to improve the situation of citrus disease management in my country*"

"*Got opportunity to work with other international level worker/experts working on HLB – new member of HLB family – was a new experience to work/see the field in Indonesia*"

"I have been exposed to the global nature of this problem. More questions have arisen in my mind such as: a) are there biotypes of the psyllid; b) are there

pathovars/races/isolates of the HLB organism; c) is the bacterium restricted to the branch of the tree where it made its entry, is so can pruning be an important component of IPM"

C Organisation - What sort of impact will the Crawford Fund training have on your organisation? Please rate the statements below

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
(1) The training will have no impact on my organisation or the work it performs	2	3	6	9	8
(2) The quality of internal training programs may improve	6	17	4	0	1
(3) The uptake of new/improved technology may increase in the organisation	4	18	6	0	0
(4) The adoption of new/improved technology by the organisation's clients (e.g. farmers or industry) may increase	4	14	10	0	0
(5) I will try to increase the quality of discussion about work	7	20	1	0	0
(6) I will try to influence the policies developed by the organisation to be more considered and well informed about potential impact	7	14	7	0	0
Average	5.0	14.3	5.7	1.5	1.5

(7) We would welcome any further comments you have about the impact the training activity may have on your organisation.

"After acquiring the technical knowledge on CTV immunoblot, detection of phytoplasmas and microscopy preparation of samples, I can start screening for CTV amongst the growers' orchards as an additional part of the role of my organisation. Screening for suggested phytoplasmas can also be carried out by my organisation. The use of simple microscopy techniques will allow my organisation to test for infection by HLB or CTV. All these diagnosis will allow my organisation to assist growers to certify that their planting material is free of CTV, HLB & phytoplasmas."

"The impact of the training is high level awareness program for the Pacific region. The Master Class program provides comprehensive basic and advanced knowledge to understand every step from the field (tree) to the lab (diagnosis). These comprehensive experience help in drawing Emergency Response plan and strategies to mitigate HLB diseased."

"I am more equipped with HLB knowledge to apply for projects in relevant field in my country and even in this region. Networks built during the HLB MC will be useful for future collaboration with my organisation."

(8) We would appreciate any other comments you have relating to the Crawford Fund, this survey, or the Master Class program you attended.

"*It is greatly appreciated that the Crawford Fund has offered this golden opportunity to meet up with scientists from many parts of the world and to and to learn from each other. Without such funding, I would not have this opportunity to share solutions to a common problem and to learn from scientists who are experts in laboratory techniques.*"

"*Having had this opportunity, I am now aware of the opportunity/facility available for young scientists. I would like to disseminate/let other family members know about it and participate/organise such master classes. I have great time. Very hands on work opportunity/facility and learn new things techniques about HLB – it will enable me to work with more confidence in the areas of citrus IPM.*"

"*The Crawford initiative is really something that needs applaud. Bringing together (representatives) of 14 countries for a HLB training is important, at the end for a better management of the disease in the region and to prevent its spread to countries where it has not been introduced. The Master Class was very well organised with full activities every day.*"

"*It is one of the best Master Class (First I attend), field trips, lab experiments, procedures which I think has enabled every participant enjoy very much. It was a real balanced class where we related what see in the field to diagnosis ie from simple tests to realtime PCR. Many thanks.*"

"*It was visionary of Crawford Fund to have conducted this training program and increased the general awareness among 16 participating countries. This will go a long way in evolving strategies to mitigate the problem.*"

Appendix 1 – Master Class Program

Sunday 20 February:

- arrive Malang (stay at Kartika Wijaya-Batu and/or Hotel Tugu-Malang for 3 nights)

Monday 21 February:

- return travel to Tlekung:
- am - Research Institute for Citrus and Subtropical Horticulture: opening meeting, then regional presentations (industry details, disease incidence and impacts, HLB mitigation strategies); presentations on the iodine starch test (IST); inspection of facilities
- pm - visits to local high altitude orchards with generally low or no HLB (iodine starch testing to be performed, 'symptoms' photographed, and samples collected for PCR and immunoblotting for HLB and CTV, and microscopy in second week)

Tuesday 22 February:

- return travel to Jember to visit low altitude HLB +ve/-ve orchards: iodine starch testing to be performed, 'symptoms' photographed, and samples collected for PCR and immunoblotting for HLB and CTV, and microscopy in second week)

Wednesday 23 February:

- travel from Kartika Wijaya-Batu and/or Hotel Tugu-Malang to Yogyakarta (stay at YogyaPlaza or Novotel for 9-10 nights)

Thursday 24 February:

- visits to orchards (including interviews with farmers) and ACIAR HORT/2000/043 (CS2/2000/043) field sites at Purworejo and adjacent localities: iodine starch testing of symptomatic plants and sampling for PCR and immunoblotting for HLB and CTV, and microscopy in second week

Friday 25 February:

- visits to orchards (including interviews with farmers) and ACIAR HORT/2000/043 (CS2/2000/043) field sites at Ngablak: iodine starch testing of symptomatic plants and sampling for PCR and immunoblotting for HLB and CTV, and microscopy in second week

Saturday 26 February:

- am - visit to Borobudur
- pm – regional presentations given by participants

Sunday 27 February:

- rest & recreation

Monday 28 February:

- am - regional presentations of symptomatology (validation of host plant identities, compilation of photographs for publication) with Indonesian presentation to include symptoms exhibited in plants in the host-susceptibility experiment;
- pm - presentations on field light microscopy & stylet tracks, followed by practical with different hosts and a range of symptom expressions of HLB and other maladies.

Tuesday 1 March:

- am - regional presentations of symptomatology continued (validation of host plant identities, compilation of photographs for publication): PCR & Elisa to determine presence/absence of HLB and CTV in tissues evaluated previously with IST and microscopy.

Wednesday 2 March:

- am/pm - continuation of laboratory work on PCR, Elisa, microscopy, stylet tracks & powdery mildew

Thursday 3 March:

- am/pm - continuation of laboratory work on PCR, Elisa, microscopy, stylet tracks & powdery mildew
- pm – continuation of laboratory work and also preparation for Friday meeting

Friday 4 March:

- am - discussions on strategies, procedures and policy requirements for implementing viable, sustainable and effective mitigation practices for HLB and other pathogens in situations ranging from small to large farms in regions with low to high potential for disease ingress
- pm - discussions on mitigation strategies

Saturday 5 March:

- depart Yogyakarta for home

Appendix 2 – Participant Details

Name	Country	Email	Institution and Address
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Appendix 3 – Master Class Certificate

Appendix 4 – Public Awareness/Media in Australia

The following lists the media coverage of the Crawford Fund Press release of 3rd March (see below):

3rd March	ABC Renmark, South Australia
	http://www.abc.net.au/rural/regions/content/201103/3156543.htm?
	National Commercial Radio Rural News
4th March	Tablelands Advertiser (Atherton)
7th March	ABC Mildura/Swan Hill, New South Wales
	http://www.abc.net.au/rural/vic/milduraswanhill/
9th March	Weekly Times
	2AD (Armidale, New South Wales: news)
	2TM (Tamworth, New South Wales: news)
	2NZ (Inverell, New South Wales: breakfast)
10th March	The Land (national)
	Queensland Country Life
	Good Fruit and Vegetables (national)

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MEDIA RELEASE

THREAT BY DEADLY CITRUS PEST BEING ADDRESSED

Attention is being given to improve biosecurity skills in neighbouring countries which will help keep Australia's citrus industry safe from the worst disease of citrus.

A devastating but exotic sounding disease of citrus including oranges, grapefruits, lemons and limes, Huanglongbing or HLB is the focus of attention at a Master Class currently being held at Gadja Madah University in Yogjakarta in Indonesia. Attended by 44 plant protection scientists from 16 countries, including Australia, and led by scientists from the University of Western Sydney and Gadja Madah University, the class is designed to develop skills in the diagnosis and management of HLB.

"Huanglongbing has earned the reputation as the worst disease of citrus that is spread by an insect," said Dr Eric Craswell, Director of Master Classes at the Crawford Fund, which is sponsoring the training with the Australian Centre for International Agricultural Research.

He explained that a tiny psyllid, or jumping plant-lice, transmits the bacteria which multiply in the plant's sap, causing yellowing of the leaves and eventual death of the trees.

"The disease is currently rife in most South and Southeast Asian countries and southern China and also in Florida and Brazil," said Dr Craswell.

"Infected trees should be destroyed to prevent spread of the infection, but farmers do not always appreciate the threat caused by retaining diseased trees."

"Another problem is that infections in alternative host trees -ornamentals such as orange jasmine – accelerate the spread of HLB."

Concern about the entry of HLB into Australia, until now free of the disease, was expressed at a February meeting of the Rural Affairs and Transport References Committee of the Australian Senate. Evidence presented by representatives of Riverina Citrus pointed to the loss of 200,000 acres of citrus in Florida, where one legislator is aiming to reintroduce legislation to provide funding to fight HLB through tariff payments made on imported citrus fruit and juice, which would raise up to USD30 million per year over the next five years. Brazil admits to removing four million trees with another two million scheduled for removal.

"Concern was also expressed to the Senate hearing that not enough was being done to address the threat and, in particular, take measures to stop the psyllid from entering Australia," he said.

"While the primary focus of our Master Class is to help plant protection experts from developing countries in Asia and the Pacific to improve their skills in the diagnosis of HLB, we recognise the significant benefits to Australia from the Master Class. Firstly, the improvement of diagnosis and management skills in neighbouring countries will reduce the spread of the disease there and thus reduce the risk that the disease will spread to Australia. Secondly, by including six Australians as participants in the class, we can help improve Australia's capacity to identify the psyllid and the disease so that any introduction can be more quickly dealt with before it spreads through our important citrus industries," he said.

"There are numerous examples of international agricultural research and extension work providing such benefits to Australia's farming industries," he concluded.

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