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www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2005, **34**, 517–524

Spread of citrus huanglongbing (greening disease) following incursion into Papua New Guinea

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Abstract. Huanglongbing (HLB) was previously known in many countries as greening disease. It is caused by the bacterium '*Candidatus* Liberibacter asiaticus' and is vectored by the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae). A delimiting survey showed that the disease had become established in, and near the border town of, Vanimo in the Sandaun Province of Papua New Guinea (PNG) by late 2002. A campaign of quarantine containment and public awareness followed. A second survey undertaken 1 year later indicated that long-distance movement of the disease and its vector had not occurred. Out of a total of 120 trees indexed by polymerase chain reaction (PCR), 4/72 were HLB-positive in 2002 compared to 12/48 in 2003. The second survey found presumptive evidence for limited HLB disease cluster expansion and further independent introduction of infected planting material. HLB-positive samples were also screened for *Citrus tristeza virus* (CTV) infection using a double antibody sandwich enzyme-linked immunosorbent assay. The symptoms observed on leaves of orange (*Citrus sinensis*) and lemon (*Citrus limon*) infected by '*Ca.* L. asiaticus' alone and '*Ca.* L. asiaticus' plus CTV were similar. This is also the first verified record of CTV in PNG. No evidence was found for the presence of HLB in four Pacific Island countries (Cook Islands, Fiji Islands, Samoa and Tonga) to the east of PNG with 18 citrus trees tested negative by PCR.

Introduction

One of the most important constraints to citrus production in much of Africa and Asia (Aubert 1990*a*) is citrus huanglongbing (HLB) disease, known previously as citrus greening disease. In South-East Asia, HLB is caused by the unculturable, phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (Garnier *et al*. 2000) and is vectored by the Asian citrus psyllid *Diaphorina citri* (Kuwayama). Long-distance spread occurs via infected and infested planting material. The disease is absent from Australia and a report of its presence in the Pacific (Fiji Islands, Tonga, Samoa and Palau) in the mid 1990s (Kiritani and Su 1999) has never been confirmed. No further evidence of the disease or the vector has been found in these or any other Pacific Island countries or territories (Secretariat of the Pacific Community Plant Protection Service, unpublished data) and undescribed DNA probes were used to detect the pathogen. Polymerase chain reaction (PCR) detection methods (Jagoueix *et al*. 1996; Hocquellet *et al*. 1999) are considered to be more specific for '*Candidatus* Liberibacter' species and became the preferred diagnostic method in later studies (Subandiyah *et al*. 2000).

In Indonesia, which neighbours Papua New Guinea (PNG) and Australia, the disease has been a severe problem for decades, particularly in orange and mandarin (Tirtawidjaja *et al*. 1965; Aubert *et al*. 1985). Towards the end of the twentieth century, the threat moved a step closer to PNG, Australia and nearby Pacific Islands when *D. citri* was reported in the north-west of the Indonesian province of Papua (formerly Irian Jaya) in 1990 (Aubert 1990*b*), then over 1000 km to the east, near Papua's provincial capital, Jayapura, in 1992 (Northern Australia Quarantine Strategy (NAQS), unpublished data, 1992). In 1999, PCR of citrus leaf samples confirmed the presence of the pathogen in these regions (Davis *et al*. 2000). *D. citri* and the pathogen then reached the town of Vanimo, Sandaun Province, PNG. The detections were made when the National Agricultural Quarantine and Inspection Authority (NAQIA), National Agricultural Research Institute (NARI) and NAQS conducted a plant health survey on the PNG side of the border in September 2002 (Weinert *et al*. 2004). This outbreak occurred in a remote and relatively sparsely populated part of PNG. In 2000, Vanimo was a town of 9778 people, with an

 10.1071/AP05059 0815-3191/05/040517

additional population in nearby coastal and inland villages of 14 515 (PNG National census data 2000). A delimiting survey conducted by NAQIA, NARI and SPC 2 months later, found that the disease was present at one more location in Vanimo and in a nearby village and that the vector was also more widespread than believed when first detected (Weinert *et al*. 2004). The current paper presents more details on the delimiting survey and describes a second follow-up survey undertaken exactly 1 year later. This information can be used to estimate the probable rates of spread of both disease and vector and the threat of spread to the rest of PNG and neighbouring countries.

This paper also reports two concurrent studies made during this period. The first concerns symptomatology of leaf samples from the outbreak in PNG. The symptoms of HLB infection are similar to nutrient deficiencies and other disorders and often a sectoral chlorosis (yellowing of one branch or one part of the canopy) seen in early stages is the clearest indication of HLB infection (P. Barkley, NSW Agriculture, personal communication, 1997). This makes the disease hard to study in the field and it is also unclear whether there is any synergistic effect of concurrent infection by the phloem-limited virus, *Citrus tristeza virus* (CTV), on HLB leaf symptom expression. CTV is a very common virus in citrus trees worldwide and tristeza symptoms have been observed previously in PNG (Philemon 1994). Anecdotal reports from south-east Asia suggest that leaf symptom expression, especially vein swelling or corkiness, increases as a result of dual infection by both pathogens. The present study investigates whether CTV and '*Ca.* L. asiaticus' interact to alter symptoms shown as compared with HLB alone. In addition, early results of an ongoing search for evidence of HLB in certain Pacific Islands lying to the east of New Guinea are reported.

Methods

Surveys in north-west Papua New Guinea

Over a period of 7 days in November 2002, a delimiting survey (survey 1) was conducted in, and near, Wewak, East Sepik Province (ESP) and in, and near, Vanimo, Sandaun Province, (SP) PNG. The survey was carried out following detection, 2 months earlier, of an infected tree in one property in Vanimo. *D. citri* was found on this and several other citrus trees in the town, as well as in one village approximately 30 km to the west. The second survey (survey 2) over 11 days in November 2003, focused on the same localities plus coastal regions of ESP and SP where local people have regular contact with Vanimo (both east and west of Wewak as far as Aitape and nearby villages). Survey 2 also included villages inland from Vanimo up to the Bewani region. Both surveys targeted domestic citrus trees in as many communities as possible and included the one ex-commercial orchard that was present in the area. Most were mature (>10 years old) non-grafted trees. Identification of citrus trees was made as far as possible from visual characters and by consultation with householders. Growers, however, were able to describe their trees only by common names (e.g. orange, lime, lemon). The corresponding species names are used in this paper, but this might be inaccurate in many cases. This is because much hybridisation has occurred within the genus, smearing the boundaries between species and many hybrids grow in the region.

Psyllid detection

At each survey location in north-west PNG, citrus and two other known host trees occasionally found in domestic properties (the citrus relative, mock orange (*Murraya paniculata*) and curry leaf (*Bergera koenigii*)) were swept with a net to determine if psyllids were present. New flush growth was common at the time of both surveys and was targeted, as this is known to be favoured by psyllids (Catling 1970). Insects were collected into 70% ethanol and returned to Port Moresby and Australia for identification.

Collection of leaf samples

At each place in north-west PNG where psyllids were found, plus sites where they were not (chosen to provide well-spaced sampling across psyllid-free zones), citrus trees showing possible HLB-like symptoms were sampled for HLB indexing. Young leaves with green on yellow vein banding, general chlorosis, chlorotic mottling, corking of leaf veins, or short upright and bushy growth habit were collected. Samples consisted of approximately 1 g fresh weight of petioles and midribs cut out of leaves that had been surface sterilised (in 1% available chlorine), chopped finely and desiccated over anhydrous calcium chloride, then frozen.

Huanglongbing screening

Samples from survey 1 were indexed for HLB by a PCR test, which detects liberibacter DNA followed by a restriction digest step to confirm the amplified DNA is from '*Ca.* L. asiaticus' and not the related pathogen, '*Ca.* L. africanus', which occurs in other parts of the world (Garnier *et al*. 2000). Testing was conducted in Australia and France, as outlined in Weinert *et al*. (2004). Samples from survey 2 were returned to Fiji for testing under Fiji Quarantine and Inspection Division permit No. 48681. Total DNA was extracted using Qiagen DNeasy plant minikits, according to the manufacturer's instructions, except for a slight modification made to improve chances of detecting the pathogen. The modification was an increase in the amount of plant tissue processed to approximately 0.25 g fresh weight, incubated in a correspondingly increased amount of buffer AP1. A slight adaptation of the PCR method of Hocquellet *et al*. (1999) was used to detect '*Ca.* L. asiaticus' DNA. In this, Platinum Taq DNA polymerase and reaction buffer (Invitrogen) were used and bovine serum albumin and W1 detergent were omitted from the reaction mix.

Effect of Citrus tristeza virus on huanglongbing leaf symptoms

Following HLB indexing for survey 2, 14 samples that gave positive PCR test results and 18 that tested negative were also screened for CTV. This was done by double antibody sandwich enzyme-linked immunosorbent assay, using an Agdia (Elkhart, IN, USA) antisera reagent set and Agdia extraction buffer, according to the manufacturer's instructions.

Incidental observations for evidence of huanglongbing in other Pacific Island countries

In addition to the survey work in PNG, HLB-like symptoms were sought during disease surveys conducted in the Fiji Islands, Samoa, Tonga and Cook Islands in 2002–2004. In these surveys, the same methods as above were used to collect samples from small numbers of citrus trees. Trees sampled were those showing either the combination of chlorotic blotch plus nutrient deficiency symptoms on leaves as observed on infected trees in PNG, or sectoral chlorosis of the canopy. The four earliest samples were tested in France at the laboratory of M. Garnier (Institut National de la Recherche Agronomique) and the remainder were tested in the Fiji Islands, as described above.

Results

The vector in north-west Papua New Guinea

Diaphorina citri was found to be widespread in the Vanimo area in both surveys, but absent from the Wewak region, over 200 km to the east (Table 1). Specimens were lodged in the NAQS entomological collection, Mareeba, Australia. Representatives were found to be identical to *D. citri* specimens from Indonesia that had been identified by D. Hollis, Natural History Museum, UK. *D. citri* was most often found on what appeared to be lemon (*Citrus limon*)

Table 1. Results of delimiting surveys for *Diaphorina citri* and huanglongbing in north-west Papua New Guinea, 2002 and 2003

Location[A]	Survey 1: November 2002			Survey 2: November 2003		
	HLB+[B]	HLB–[B]	*D. citri*[C]	HLB+[B]	HLB–[B]	*D. citri*[C]
			Vanimo			
<200 m from IF1	2	8	Y	5	3	Y
200–700 m from IF1		8	Y	2	8	Y
<500 m from IF2	1		Y		3	Y
>700 m from IF1		27	Y		3	Y
			Lido			
<500 m from IF3	1		Y	4		Y
			Coast west of Vanimo			
Wutung		2	Y		1	N
Musu		2	N			
Yako		2	N			
Waramo		2	Y		1	Y
			Coast east of Vanimo			
Warrabris		1	N			
Pasi		2	N		1	N
Wusipi		1	Y		1	Y
Waterstone		1	N		1	Y
Dapu		1	Y		1	N
Blackwater				1		N
Onei					1	N
			Inland from Vanimo			
Bewani					1	N
Amoi					1	N
Ituly					1	N
			Aitape region			
Aitape					3	N
Namanbris					1	N
Wagan					1	N
			Aitape-Wewak road			
Yagamul					1	N
Suain					1	N
Umamon					1	N
Wewak		11	N		1	N
Total for survey	4	68		12	36	

[A]IF1: Infection focus 1, where the first HLB-positive tree was detected in Vanimo in September 2002.
IF2, IF3: Infection foci 2 (over 1 km from IF1 in Vanimo) and 3 (Lido village), identified from results of survey 1.
[B]Number of trees positive (+) or negative (−) in polymerase chain reaction diagnostic tests conducted in the Northern Australia Quarantine Strategy laboratory, Australia or the Institut National de la Recherche Agronomique laboratory, Bordeaux, France (survey 1) and in the University of the South Pacific's Institute of Applied Science, Fiji Islands (survey 2).
[C]*Diaphorina citri* found (Y) or not found (N) after sweeping sampled or nearby trees.
In the region within 700 m of IF1, many trees were sampled on survey 1 and most accessible trees were sampled on survey 2. Elsewhere, the trees tested were 1 of usually more than 10 growing at each location. Data from survey 1 has been summarised in Weinert *et al*. (2004).

trees and two *M. paniculata* were found heavily infested with *D. citri*. This is an ornamental host favoured by *D. citri* that is not common in that part of PNG. In survey 1, *D. citri* was detected at several, but not all, locations on the coast west and east of Vanimo. *D. citri* was also found in almost all parts of Vanimo town. After 12 months (survey 2), the most easterly location where psyllids were seen was still the Wusipi region, approximately 10 km east of Vanimo. However, the insects were not found at two locations where they were previously recorded in survey 1. One location near Wusipi that was free of psyllids in survey 1 had now become infested.

The disease in north-west Papua New Guinea

Possible HLB disease symptoms were hard to see as many trees were suffering from long-term poor nutrition and neglect. Four trees in survey 1, (apparently one lime (*Citrus aurantifolia*) and three lemons) and 12 more trees in survey 2 (two cumquat (*Fortunella japonica*) and what appeared to be six lemon and four orange (*Citrus sinensis*)) tested positive for '*Ca.* L. asiaticus' (Table 1). Negative results were obtained from a total of 104 other trees over both surveys (Table 1). These were mostly cumquats and what was thought to be lemons and oranges plus a few pummelo (*Citrux maxima*), lime and mandarin (*C. reticulata*) and some unknown *Citrus* spp. The principal symptom shown by trees that were HLB-positive was chlorotic blotching of most leaves. Of the four infected trees identified in survey 1, three were revisited in survey 2 (the fourth had been destroyed) and were retested and all gave positive results (this data not included in Table 1). The known diseased trees had declined noticably, with extensive yellowing of foliage and little or no fruit set. Many fruits were present on these trees when observed on survey 1.

Of the four HLB-positive trees found in survey 1, two were on domestic properties immediately adjacent to the back yard where the first infected tree was found two months before (infection focus 1 (IF1)) (Table 1). Another was in a domestic property over 1 km from that location (infection focus 2 (IF2)), and the fourth was on a public lawn in the village of Lido, approximately 4–5 km to the west of Vanimo (infection focus 3 (IF3)). Over ten trees growing in areas between these locations tested negative for HLB in survey 1. In survey 2, HLB was detected in more trees within Vanimo near IF1 and in Lido (Table 1). Importantly, HLB was also confirmed in another new location far from these known infection foci, on what appeared to be a lemon tree found at Blackwater over 10 km to the east of Vanimo. Nucleic acids from this sample were extracted and tested again, giving a second positive result.

Key survey 2 results around IF1, IF2 and IF3

Around IF1, there are areas of public land where no citrus is grown and there are also streets of domestic properties that contain citrus trees. Approximate distances from IF1 to the trees that were sampled on survey 2 were calculated using Global Positioning System locations. This revealed new infections (HLB-negative in survey 1, HLB-positive in survey 2) in three trees within 50 m of IF1 (Fig. 1). Distribution in the area within 50 m of IF1 was relatively patchy. Two trees in the same back yard as a new infection plus one between the new infection and IF1 tested negative for HLB. The next most distant trees, approximately 150 m to the north-west and 100 m to the south of IF1, were positive for HLB, but these trees were not sampled in survey 1. Three more trees, approximately 200, 500 and 1000 m to the north-west and one tree approximately 400 m to the south, were negative. The first available citrus tree south-east of IF1 was approximately 300 m away and was also a new infection, testing HLB-positive in survey 2 after being negative in survey 1. When all available trees were sampled in the north-east direction, the first two trees, at approximately 300 m, were HLB-negative, but the next property around 600 m out, contained one HLB-positive tree that was not sampled in survey 1 and two trees that tested negative.

In contrast, no evidence was found for spread of HLB at IF2. In the same domestic property as the IF2 tree, one tree sampled, plus two more within 50 m and 200 m, respectively, tested negative for HLB. At Lido (IF3), many trees were found throughout the village showing possible symptoms of advanced HLB. A selection was sampled and all were HLB-positive.

Effect of Citrus tristeza virus on huanglongbing leaf symptoms

Overall, CTV was present in 21/32 samples tested. Of the 14 HLB-positive samples screened for CTV infection, eight (two cumquat and apparently three lemon, two orange and one lime) were also positive for CTV and six (thought

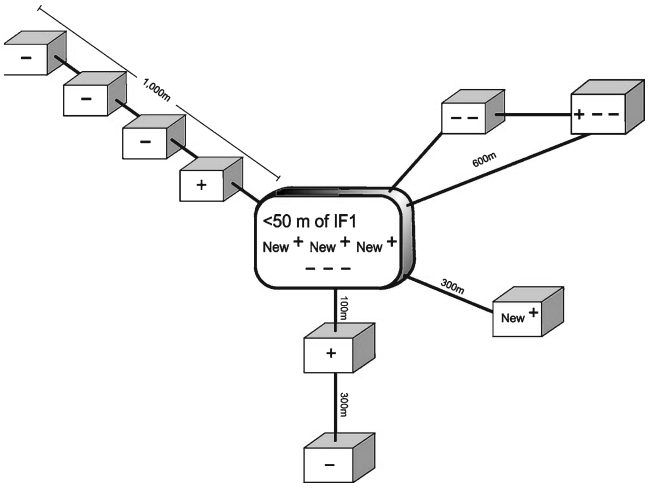

Fig. 1. Diagrammatic representation of huanglongbing disease occurance around IF1 in central Vanimo, November 2003. –, citrus tree that tested negative for '*Ca.* L. asiaticus' by polymerase chain reaction (PCR); +, citrus tree that tested positive for '*Ca.* L. asiaticus' by PCR; New+, citrus tree that tested negative for '*Ca.* L. asiaticus' by PCR in November 2002, then positive in November 2003. Approximate distances (m) represented by straight lines.

to be four lemon and two orange) tested negative (Table 2). The principle symptoms shown by five of the dually infected samples (two orange, two cumquat and one lemon) and also five of the samples infected with '*Ca.* L. asiaticus' alone (three lemon and two orange), plus one of the samples infected with CTV alone (apparently a lemon) were chlorotic blotch plus swollen or corky veins. General chlorosis together with other nutrient deficiency symptoms, a green on yellow vein banding, were shown by three of the dually infected samples (two lemons, one lime), one sample that tested positive only for HLB (a lemon) and all remaining samples that tested positive only for CTV (six cumquats and what was thought to be three oranges, two lemons and one lime). Examples of symptoms shown by what appeared to be orange leaves infected with '*Ca*. L. asiaticus' only, '*Ca*. L. asiaticus' plus CTV, and CTV alone are shown in Fig. 2.

Incidental observations for evidence of huanglongbing in other Pacific Island countries

No convincing evidence of ongoing HLB epidemics, such as spreading decline problems or aggregated groups of trees showing HLB-like symptoms, was found on the surveys conducted elsewhere. A total of 18 trees, four on Viti Levu Island in Fiji, four on Savai'i Island in Samoa, two from Ha'apai and three from Vava'u in Tonga, plus two on Rarotonga and three on Aitutaki in the Cook Islands were sampled and all tested negative for HLB.

Discussion

In north-west PNG in November 2002 (survey 1), *D. citri* numbers were clearly higher and detectable populations were more widely distributed compared with that found in

Table 2. Symptoms shown by citrus leaves tested for huanglongbing (HLB) and *Citrus tristeza virus* (CTV)

No. samples (tree type)[C]	Symptoms
	HLB[A] *and CTV*[B]
5 (2 × orange, 2 × cumquat, 1 × lemon)	Chlorotic blotch and corky veins
2 (1 × lemon, 1 × lime)	General chlorosis and several nutrient deficiency symptoms
1 (lemon)	General chlorosis and green on yellow vein banding
	HLB[A] *only*
5 (4 × lemon, 1 × orange)	Chlorotic blotch and corky veins
1 (lemon)	Chlorotic blotch only
	CTV[B] *only*
8 (4 × cumquat, 3 × orange, 1 × lime)	Chlorotic blotch only
1 (lemon)	Chlorotic blotch and corky veins
1 (cumquat)	Chlorotic blotch and green on yellow vein banding
1 (cumquat)	Chlorosis and green on yellow vein banding
1 (lemon)	Chlorotic blotch and vein clearing
1 (lemon)	Vein clearing and green on yellow vein banding

[A]Tested positive for '*Ca.* L. asiaticus' by polymerase chain reaction.
[B]Tested positive for *Citrus tristeza virus* by double antibody sandwich enzyme-linked immunosorbent assay.
[C]Identifications of trees (other than cumquat) were approximate, based on opinions of growers and/or visual characteristics available.

Fig. 2. Leaves of what appeared to be orange trees that indexed positive for (*A*) '*Ca.* L. asiaticus' alone, (*B*) '*Ca.* L. asiaticus' plus *Citrus tristeza virus* (CTV), and (*C*) CTV alone when tested for these two phloem-infecting pathogens.

September 2002 (Weinert *et al*. 2004). This might have been because of a period of dry weather followed by the first growth flushes of the new wet season. Such conditions promote increases in population of *D. citri* (Catling 1970; Aubert *et al*. 1985). Between survey 1 and 2, the psyllid had apparently not spread very far even though no insecticides were known to have been sprayed on any trees. *D. citri* was not found at two locations in survey 2 where it was previously found in survey 1. This suggests that populations might not have been as high in survey 2, making detection more difficult. However, survey 2 found evidence for some movement of psyllids into a new location. Importantly, there appears to have been no movement of *D. citri* inland from Vanimo, along the Bewani road.

As symptoms of HLB can be similar to other disorders such as nutrient deficiency, the disease can be difficult to detect in unthrifty trees. Chlorotic blotching and swollen or corky leaf veins were found to be two key visual guides, especially when they occurred in combination. The HLB–CTV interaction study reported here suggests that CTV infection has little effect on HLB leaf symptoms shown by what were apparently mature, flushing orange and lemon trees in, and near, Vanimo. A group of these trees was identified in which some were infected by '*Ca*. L. asiaticus' alone and some by this pathogen together with CTV. All showed similar chlorotic blotch and corky veins, suggesting no synergistic interaction is necessary for these symptoms to become prominant. In fact, the most extreme example of vein swelling or corkiness was found on what appeared to be an orange tree that was indexed HLB-positive only (Fig. 2). Using electron microscopy, Chen *et al*. (1972) showed that individual phloem cells of CTV-tolerant trees infected with both '*Ca*. L. asiaticus' and CTV rarely contained both pathogens. They conclude that there is no histological or cytological evidence for increased symptom severity following dual infection. However, the effect of CTV could depend on host tolerance. Oranges and lemons are both susceptible to CTV (see http://image.fs.uidaho.edu/vide/sppindex.htm), but reactions vary depending on the CTV strain involved (Garnsey 1999). Experiments using artificial inoculation might help in future investigation of this interaction. The present study also provides the first published laboratory test record confirming the presence of CTV in PNG.

Short distance spread of HLB is thought to mostly occur when psyllids are disturbed and they react by jumping and making short landing flights of 3–5 m (Aubert 1990*b*). However, psyllids can move 25–30 m when disturbed (Gottwald *et al*. 1991*b*), and further distances if they rise up above the tree canopy and are carried in the wind (Aubert 1990*b*).

Aggregation of diseased plants is a characteristic of HLB epidemics in citrus orchards (Gottwald *et al*. 1989, 1991*a*, 1991*b*). Clustering of HLB-infected plants was evident in Vanimo town, where 7 infected trees were found within 700 m of IF1 on survey 2. Survey 2 also provided presumptive evidence of disease focus expansion. Three HLB-negative trees within 50 m of IF1 changed to positive during the 12 months between surveys. There was also one outlying new infection possibly also from this source at around 300 m (Fig. 1). These trees might have actually contained undetectable levels of the pathogen during survey 1 as the latency period can last from 6 to 12 months (T. Gottwald, USDA, Florida, personal communication, 2003). As the two HLB-positive trees at 100 m and 150 m away from IF1 were not sampled on survey 1, it is not known if these are also the result of spatial expansion from IF1 or of independent introduction as infected planting material. Between IF1 and the HLB-positive tree 600 m from IF1, two trees remained HLB-negative and infested with *D. citri* throughout. This suggests that psyllid-borne tree-to-tree spread across the 600 m had not occurred. The limited spread of HLB observed over one year in Vanimo town contrasts greatly with HLB epidemiology in commercial production situations in Asia. Incidence of HLB in mandarin crops in China reaches such high levels that entire orchards can lose their commercial value after 7–8 years (Aubert 1990*c*). Gottwald *et al*. (1991*b*) document HLB spread in a commercial mandarin planting of 20 000 originally disease-free trees in China. They found that the number of diseased trees increased from 16 to 2880 over a 2-year period.

After survey 1 was completed, the possibility of eradication of psyllid and disease from PNG was considered and rejected, mostly because of concerns for public health. The destruction of infected and all surrounding citrus trees within at least 50 m was proposed. This destruction zone is more than double the size of known HLB disease clusters measured on both Réunion Island, which were 12 m × 24 m (Gottwald *et al*. 1989), and in the Philippines, which were 20 m × 20 m (Gottwald *et al*. 1991*a*). Prior to felling trees, large quantities of insecticide would need to be applied to all back yard psyllid host trees in these 50 m zones. Such exposure of householders to toxic chemicals seemed unjustified as similar incursions are likely to follow. This is because HLB is present just to the west (Davis *et al*. 2000), making new cross-border introductions of infected and infested planting material and/or wind blown insects probable.

The two new locations where HLB was found in survey 1 (IF2 in Vanimo and IF3 in Lido) were, respectively, approximately 1 and 5 km from IF1. It is quite likely that independent introduction(s) had occurred, because in between the foci of infection many psyllids but no disease was found. The results of survey 2 also provide evidence of further independent introduction of at least 1 infected tree. The tree at Blackwater, more than 10 km from Vanimo, was not recently planted and was located several kilometres from the nearest place where psyllids were detected in survey 2. This suggests

that an infected tree had been planted. Blackwater is a region populated by refugees from Papua, so the tree might have been brought across the border.

From early 2003, a campaign of quarantine containment in the Vanimo region and public awareness using posters and radio broadcasts was carried out across PNG. This was instigated by NAQIA in collaboration with SPC. Legislation was set up to prevent movement of planting material of citrus and the favoured psyllid host (*M. paniculata*) from the Vanimo region and SP. Such action might be effective as marcotting is not a common practice within PNG, and road and air linkages to the rest of PNG are limited. Comparision of results from surveys 1 and 2 demonstrates that natural spread rates are very low. Therefore, successful containment of the disease is possible. If movement of pathogen and vector by humans could be prevented, then possible slower spread through natural and domestic vegetation between citrus trees might be the principle threat. Although little is known about which other plant species might be hosts for '*Ca*. L. asiaticus', *D. citri* can feed on many plant species (Aubert 1990*b*). Psyllid hosts listed by Aubert (1990*b*) that are known to occur in PNG include *M. paniculata* and *Triphasia trifolia* (see http://plantnet.rbgsyd.nsw.gov.au/PNGplants) and also *B. koenigii* (NAQS unpublished data, 1998). In addition, certain *Clausena* spp. and *Atalantia* spp. are included in the listing of Aubert (1990*b*). Unidentified *Atalantia* spp. and different *Clausena* spp. are known to be present in PNG (see http://plantnet.rbgsyd.nsw.gov.au/PNGplants).

The limited HLB screening results reported here from ongoing surveillance conducted elsewhere in the Pacific adds some support to the widely held opinion that HLB is not present as an established disease problem in these countries. If the disease was once introduced to Tonga, Samoa and Fiji Islands before the mid 1990s as suggested by Kiritani and Su (1999), then this must have happened without concurrent introduction of a vector insect as no known HLB epidemics resulted. It is likely that other Pacific Island countries and territories are similarly HLB-free as there are no records of *D. citri* on any Pacific Island other than New Guinea. If this is so, then the importance to neighbouring Pacific Island nations as well as Australia, of successful containment within PNG is heightened.

Acknowledgements

Most of this work was undertaken by the Secretariat of the Pacific Community's (SPC) Plant Protection in the Pacific Project, which is funded by the European Union. The Australian Quarantine and Inspection service provided identifications free of charge following survey 1 and partially funded survey 2. B. Aalbersberg of the University of the South Pacific's Institute of Applied Science, Suva, Fiji Islands is gratefully thanked for allowing use of a molecular diagnostics laboratory to analyse samples from survey 2. The laboratory of the late M. Garnier, Institut National de la Recherche Agronomique, Bordeaux, France is gratefully thanked for testing 4 citrus samples. S. Tukidia, SPC is thanked for preparation of Figs 1 and 2.

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Received 11 November 2004, accepted 2 June 2005