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Aspects and Insights of Australia-Asia Collaborative Research on Huanglongbing

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Abstract

We highlight current Australia-Asia collaborative research on the development of sustainable management practices for minimising the impact of huanglongbing and its vector in Asia, the Asiatic citrus psyllid *Diaphorina citri* Kuwayama, and pre- and post-incursion management plans for the Australian citrus industry. Our research commenced in the mid 1990s as part of projects on the integrated control of citrus pests in China, Malaysia, Thailand and Việt Nam. Our current focus is on collaborative research in Indonesia and Việt Nam. We are also linked to research in China, Malaysia and Pakistan. Our research activities include studies on host plants (including *Citrus* relatives), natural enemies, mineral nutrition, environmental factors, and vector control options including mineral oils, imidacloprid, rotation plantings and guava interplants. We summarise and discuss past results, initial results from current studies, and insight into the possible impact of hot climates on the seasonal incidence of the pathogen in plant host canopies and vector populations. We express concerns about the currently perceived origins and number of *Candidatus* Liberibacter species that cause huanglongbing. Lastly, we discuss the origins and systematics of the genus *Citrus* and its relatives within the Rutaceae subfamily Aurantioideae, host susceptibilities to both the pathogen and its vectors, and germplasm conservation.

Introduction

Huanglongbing (黃龍病) is the official name of a serious disease that can destroy poorly managed citrus orchards in Asia within a few years of planting (da Graça 1991; van Vuuren 1996; Bové 2006). Literally 'yellow dragon disease', it has a number of common names and is most widely known as citrus greening (da Graça 1991; Halbert & Manjunath 2004). It is caused by phloem-limited, Gram-negative liberibacters (α -Proteobacteria) in Asia, Papua New Guinea and the United States of America by *Candidatus* Liberibacter asiaticus (Jagoueix et al. 1994; Garnier et al. 1996; Weinert et al. 2004; Gottwald et al. 2006); in Africa by *Ca. L. africanus* (Jagoueix et al. 1994); in Mauritius and Réunion by *Ca. L. asiaticus* and *Ca. L. africanus* (Halbert & Manjunath 2004); and in Brazil by *Ca. L. asiaticus* and *Ca. L. americanus*, the third known liberibacter associated with the disease (Teixeira et al. 2005a, b). A sub-species, *Ca. L. africanus* subsp. *capensis*, has been recorded in Cape chestnut (*Calodendrum capense* (L. f.) Thunb. (Rutaceae: Rutoideae), an ornamental tree in Southern Africa (Garnier et al. 2000). In Asia, Papua New Guinea, Florida and Brazil huanglongbing is transmitted by the Asiatic citrus psyllid, *Diaphorina citri* Kuwayama [Hemiptera: Psyllidae] (Capoor et al. 1967; Martinez & Wallace 1967; da Graça 1991; Waterhouse 1998; Halbert & Manjunath 2004; Weinert et al. 2004; Teixeira et al. 2005b). In Africa, the African form of the disease is transmitted by the African citrus psyllid, *Trioza erythrae* (del Guercio) (Triozidae) (McClellan & Oberholzer 1965; da Graça 1991). In Réunion, *Ca. L. asiaticus* and *Ca. L. africanus* may possibly be transmitted by both psyllids (da Graça 1991; Halbert & Manjunath 2004). The disease does not occur in Australia (Bellis et al. 2005) and is widely assumed to have originated in China (da Graça 1991; Waterhouse 1998; Bové 2006). It is also widely considered that *Citrus* originated in Southeast Asia between India and China and southwards through Malesia (Swingle & Reece 1967; Webber et al. 1967; Dugo & Di Giacomo 2002).

In this paper we highlight aspects of Australia-Asia collaborative research on citrus pests and diseases, including *D. citri* and huanglongbing (HLB) and raise issues we think require debate and resolution.

Initial ACIAR-funded research

From 1993 to 2000, the Australian Centre for International Agricultural Research (ACIAR) funded two projects (ACIAR CS2/1993/005 & CS2/1996/176), the first in China and the second in China, Thailand, Việt Nam and Malaysia. These projects led to several publications on the control of citrus pests, including two on *D. citri* (Rae et al. 1997; Leong et al. 2002). Rae et al. (1997) reported studies that showed that deposits of dilute sprays of aqueous mineral oil emulsions suppressed oviposition by adult female *D. citri*, and that sprays also had significant impacts on the mortality of nymphs and eggs. Leong et al. (2002) reported the impact of multiple applications of either 0.3% or 0.5% (v/v) sprays on the psyllid and other pests, and on some foliar pathogens in citrus orchards in Sarawak, East Malaysia. Comparisons were made with two spray programs based on the use of synthetic pesticides, including foliar applications of imidacloprid. All control programs were similarly effective against *D. citri*, citrus leafminer (*Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae]), armoured scales (including red scale (*Aonidiella aurantii* (Maskell) and mussel scale (*Lepidosaphes beckii* (Newman) [Hemiptera: Diaspididae]), and whitefly (*Aleurocanthus* sp. [Hemiptera: Aleyrodidae]). However, the mineral oil program was not as consistently effective against black citrus aphid (*Toxoptera citricida* (Kirkaldy) [Hemiptera: Aphididae]) and citrus mealybug (*Planococcus citri* (Risso) [Hemiptera: Pseudococcidae]) as the pesticide programs, but it was more effective than these programs against chilli thrips (*Scirtothrips dorsalis* Hood [Thysanoptera: Thripidae]), citrus red mite (*Panonychus citri* (McGregor) [Acari: Tetranychidae]) and citrus rust mite (*Phyllocoptruta oleivora* (Ashmead) [Acari: Eriophyidae]). All programs were based on fortnightly, sometimes weekly, applications of sprays. These programs were excessive and it has been an aim of our research to reduce the frequency of spraying by focusing on application of sprays to growth flushes, primarily to control *D. citri* and citrus leafminer, and knowing that these sprays also control other pests and some diseases. The results of this ACIAR-funded research led to publication of extension and technical literature on the use of mineral oils in citrus integrated pest management (IPM) programs, including two recent books in Chinese (Huang et al. 2005; Rae et al. 2006).

Current ACIAR-funded Research

Current research is based in Indonesia at Universitas Gadjah Mada, Yogyakarta, Central Java, the Indonesian Research Institute for Citrus and Subtropical Fruits (IRICSF), Batu, East Java, and in Việt Nam at the Southern Fruit Research Institute (SOFRI), My Tho, Tien Giang, and at the National Institute for Plant Protection (NIPP), Ha Noi, and commenced in January 2003. Our initial objectives included research on: host plant distributions and host plant susceptibility to the pathogen and to *D. citri*; natural enemies of the vector; feeding behaviour of the vector; the impact of plant mineral nutrition on the disease; environmental factors, particularly those related to increasing altitude on ingress and spread of the vector and the disease into orchards; and vector control options including mineral oils, imidacloprid, rotation plantings and guava interplants. Work on germplasm selection and major field trials on the impact of altitude and latitude did not proceed as planned in Việt Nam. As a result of these studies, resolution of concerns about the systematics of the Aurantioideae, and the origins the genus *Citrus* and the genus *Liberibacter* have become a major focus of our research. Major field trials on host plants, influences of altitude (40 m, 670 m and 1300 m asl) and vector control options commenced in Java in late 2005, with the planting of pathogen-free trees and a selection of host plants of *D. citri*. Observations by SOFRI staff (see below) have led us to incorporate studies on the impact of guava (*Psidium guajava* L. [Myrtales: Myrtaceae]) on the incidence of *D. citri* and HLB in citrus orchards into the research program. We are also investigating the possible impact of air pressure on the abundance of *D. citri* to determine if declining pressure limits development of populations at and above 600 m asl.

Host plants. The host-plant field trial is located at Purworejo (105° 11' E, 5° 10' S, 60 m asl) near Yogyakarta in Java. It was established in October 2005 and currently comprises 20 known or potential hosts of *D. citri*: within the Aurantieae (Citreae); *Aegle marmelos* (L.) Corr., *Citrus × aurantiifolia* (Christm.) Swingle, *C. × aurantium* L. 'Natsudaidai', *C. hystrix* DC, *C. × junos* Siebold ex Tanaka (yuzu or Japanese citron), *C. maxima* (Burm.) Merr., *C. reticulata* Blanco 'Keprok Grabag', *C. reticulata* 'Leter', *C. reticulata* 'Siem', *Feroniella lucida* (Scheff.) Swingle, *Limonia acidissima* L., *Murraya exotica* L., *M. paniculata* (L.) Jack, *Swinglea glutinosa* (Blanco) Merr., and *Triphasia trifolia* (Burm. f.) P. Wilson.; within the Clauseaneae, *Bergera koenigii* L., *Clausena harmandiana* (Pierre)

Pierre ex Guillaumin, *Cl. lansium* (Lour.) Skeels, and *Glycosmis pentaphylla* (Retz.) DC. There are 16 replicates: a total of 320 plants. Sampling commenced October 2005 and will continue until late 2008. From October 2005 to July 2006, adult psyllids were most abundant on *C. × junos*, then *S. glutinosa* and *M. exotica*. During the same interval nymphs were most common on *Citrus hystrix*, *M. exotica*, *S. glutinosa* and *B. koenigii*. More than 240 adults and 420 nymphs were recorded over the 9 months. At this point, *M. paniculata*, as a host of adults, ranks thirteenth among the 20 plant species and varieties in the trial, and no nymphs have been recorded on any of the 16 *M. paniculata* plants in the trial. These results were unexpected, as *M. paniculata* is considered to be the favoured host of *D. citri* (da Graça 1991). However, we are cognisant that host preferences may change as the plants mature. In addition to this trial, a smaller trial comprising *C. inodora* F. M. Bailey, *C. × virgata* Mabb. 'Sydney Hybrid', *Cl. anisum-olens* Blanco, *Citropsis gabunensis* (Engl.) Swingle & M. Kellerm., *Ci. gilletiana* Swingle, *Ci. schweinfurthii* (Engl.) Swingle, *Naringi crenulata* (Roxb.) Nicolson and *Severina buxifolia* (Poir.) Tenore is being established at Purworejo.

With respect to *M. paniculata*, we are not certain if we are dealing with a highly variable species (*M. paniculata*), the latter and a hybrid (*M. exotica*), or two species (*M. paniculata* and *M. exotica*), as the validity of *M. exotica* as a species is uncertain (see Stone 1995a; Mabblerley 1998; Ranade 2006). However, in our studies, significant differences in morphology and molecular biology have been detected between the two species as named using RAPD analysis (Inggit Puji Astuti, UGM, pers. comm.), and, at this point, *M. exotica* is growing and flowering more prolifically in the field than *M. paniculata*. Symptoms of huanglongbing have also been observed in *M. exotica* following confirmed inoculation with *Ca. L. asiaticus* (Siti Subandiyah & Achmed Hamiwan, UGM, pers. comm.). Li & Ke (2002) reported detection of the pathogen in *M. paniculata*, but we consider it possible that the plants used in their study were *M. exotica*. Likewise, recent extensive records of *M. paniculata* as a host of *Ca. L. asiaticus* and *Ca. L. americanus* in Brazil (Lopes et al. 2006) may actually pertain to *M. exotica* (Silvio Lopes & Andrew Beattie, pers. discussions, December 2006). Most *Murraya* observed by one of us (GACB) with colleagues in Guangzhou, Guangdong, resemble *M. exotica* as described by Stone (1985a), and plants sent from the Xishuangbana Botanic Gardens in Yunnan to Indonesia for our studies were, although being labelled *M. paniculata*, closely resembled *M. exotica* according to their morphology and molecular biology. Plants from the University of California, Riverside, again labelled *M. paniculata* were also found by the same methods to be *M. exotica* (Inggit Puji Astuti, UGM, pers. comm.). We suspect that many host records for *D. citri* in China that have been ascribed to *M. paniculata* (see Yang et al. 2006) should have been recorded as *M. exotica*. Phytochemical differences between the two forms were reported by Li et al. (1988). On the basis of molecular studies on the genus ('sensu lato'), Ranade et al. (2006) concluded that *M. exotica* is a synonym of *M. paniculata*—but they presented no data to support this conclusion and they did not use the OPN19 primer. Clearly, there is an urgent need to resolve the status of *M. exotica* and, if it is a hybrid, its parentage. We are working to resolve these issues. Our prime concern is that under field conditions, one or both 'forms' may be asymptomatic hosts of *Ca. L. asiaticus* either permanently after infection or temporarily. Accurate classification of the species of *Murraya* has important implications for management of HLB, and for the development of incursion management plans for Australia and other countries where the disease and *D. citri* do not occur, particularly as *Murraya* is a popular ornamental shrub and is widely moved in the nursery trade.

Natural enemies. Four species of predatory coccinellids [Coleoptera: Coccinellidae] have been observed on citrus trees in central Java: *Coelophora inaequalis* (Thunb.), *Cheilomenes* (= *Menochillus*) *sexmaculata* (Fabricius), *Harmonia* sp. and *Cryptogonus orbiculus* Schönh. Initial results (Tris Haris Ramadhan, UGM, pers. comm.) suggest that their abundance is influenced by interactions between climate (altitude), tree type, tree phenology and the abundance of prey. Initial laboratory studies with *Ch. sexmaculata* have focused on larval consumption of *D. citri* and *T. citricida*. In choice tests, there was no preference for either prey species, but results from other studies suggest that the beetle develops faster, has a higher fecundity and survives better on *T. citricida*.

Natural enemies are important regulators of the vast majority of phytophagous arthropods. In most instances, the relevance of natural enemies to the control of the psyllid vectors of HLB is limited as it is axiomatic that their survival depends on the presence of their hosts and that the presence of hosts infers transmission of the disease (see Yang et al. 2006). This conundrum is exacerbated by the number of hyperparasitoids of the two primary parasitoids of the psyllids, particularly *D. citri* (see

Waterhouse 1998). Nevertheless, control measures, within the context of minimising the incidence of the disease, should seek to optimise the effectiveness of the natural enemies. However, there is scant knowledge about the effectiveness of psyllid predators in Asia (see Yang et al. 2006). This, given observations on the important role of predators of *D. citri* in Florida (Michaud 2004), is an area of research that warrants further study in the region.

Mineral oils and other strategies for managing *Diaphorina citri*. Trials are being conducted at sites in Central Java and southern and northern Việt Nam. Six treatments are being compared at each site: (1) an *n*C21 horticultural mineral oil (HMO) and conventional fungicide program for initially pathogen-free trees (PFTs), (2) an *n*C24 agricultural mineral oil (AMO) and conventional fungicide program for PFTs, (3) a conventional synthetic pesticide program with PFTs, (4) an imidacloprid (initially as a soil drench then as trunk applications), conventional fungicide and acaricide program for PFTs, (5) a conventional fungicide program, incorporating low dose applications of abamectin and HMO as required for control of citrus leafminer, for initially PFTs, and (6) as for the latter, but with trees of unknown pathogen status. The last two treatments are also being used to assess the potential for maintaining yields per hectare and minimising pesticide use by replacing diseased trees within orchards on a regular basis or through rotation plantings of citrus orchards with other crops (e.g. rice or other fruit trees). Psyllid populations at all sites have been too low to obtain meaningful results. This stems, in part, from the late planting of trees in Central Java and northern Việt Nam, and from the unexpected impact of guava interplants on psyllid populations and the incidence of HLB at the project site in southern Việt Nam. The effects of guava interplants at latter site, and in nearby orchards, are discussed in greater detail at the end of this section.

Although we have no data as yet from our field sites in Indonesia and Việt Nam, some data has been obtained for imidacloprid and minerals oils in laboratory and greenhouse studies. The studies with imidacloprid were conducted at NIPP and focused on the longevity of psyllid adults after application of the systemic pesticide as a soil drench (three treatments involving application of 50 mL of 0.2%, 0.4% or 0.6% (w/v) solutions per potted two-year old tree). Levels of mortality fell as the number of weeks after application of the chemical treatments increased. Some 9-20% of adults were still alive 24 hours after feeding on plants treated two weeks earlier and 17-32% of adults were still alive 24 hours after feeding on plants treated 10 weeks earlier (Pham Van Lam et al., pers. comm.). Given that infective *D. citri* adults can transmit the pathogen shortly after feeding on a plant (Capoor et al. 1974), it is clear that frequent applications (with frequency dependent on dose) of the chemical would be required to prevent transmission. Such use of systemic pesticides applied as soil drenches, or to the trunks of trees, might be very effective for 1-2 years after planting. However, it is unlikely to be sustainable for several reasons. These include: (a) development of resistance in the psyllid and in aphids, scales, mealybugs, thrips and citrus leafminer; (b) cost (of genuine product); and (c) residues in mature fruit. Estimating dose accurately also requires knowledge of likely influences of environmental conditions, soil characteristics, and tree size, growth rates and phenology.

Studies with an *n*C24 AMO (SK EnSpray 99) have been conducted at NIPP and UGM, and some studies have been conducted with an *n*C21 HMO (Sunspray Ultra-Fine) at NIPP (Pham Van Lam and Motif Eko Poerwanto, pers. comm.). Choice and no-choice tests at UGM with mandarin, sweet orange (*C. × aurantium* L.) and *M. paniculata* have shown that deposits can significantly reduce feeding and landing by adults, oviposition, egg hatch and survival of first instar nymphs. These effects were generally significantly different from controls for deposits of $\geq 0.5\%$ v/v aqueous emulsions applied as sprays or by dipping. Most impacts of deposits of $\geq 0.5\%$ oil led to $> 50\%$ reductions for each parameter: some were as high as 94%.

Current recommendations for using mineral oils for control of *D. citri* and citrus leafminer in Southeast Asia are summarised as follows: (1) apply sprays to spring, summer and autumn flush growth; (2) start spraying as buds begin to open in each flush cycle; (3) apply up to four sprays per cycle at 7 to 10 day intervals until most leaves within the cycle are longer than 10 mm; (4) use 0.4% to 0.5% v/v oil for the first two sprays in each cycle; (5) apply these sprays thoroughly to mature leaves, buds and immature leaves; (6) use 0.25% v/v oil for the third and subsequent sprays in each cycle and apply these sprays thoroughly to buds and immature leaves, but not to mature leaves This program will simultaneously control most mites, scales, whiteflies, citrus leafminer, citrus greasy spot (*Mycosphaerella citri* Whiteside [Dothideales: Dothideaceae]), black mildew (*Meliola citricola* H & P Sydow [Meliolales: Meliolaceae]), citrus powdery mildew (*Oidium tingitaninum* CN Carter

[Erysiphales: Erysiphaceae]), algal spot (*Cephaleuros virescens* Kunze [Trentepohliales: Trentepohliaceae]), sooty mould fungi, and foliicolous lichenised fungi (Leong et al. 2002). Efficacy may be improved by the addition of 0.03% to 0.05% imidacloprid to the oil sprays, but this should not be necessary if imidacloprid or similar insecticides are applied as soil drenches or to tree trunks between flushing cycles.

HMOs and AMOs are safe for humans to use and have virtually no significant impacts on natural enemies. There has been no reported instance of resistance to these oils or their less refined predecessors in over 100 years of use. If used as recommended, they are rarely phytotoxic: only products that conform to recognised standards (Beattie et al. 2002) and sold by reputable retailers should be used. Mineral oils should be used cautiously on plants known to be more susceptible than others to phytotoxicity (e.g., mandarins are generally more susceptible than oranges). They should not be applied to moisture stressed trees (trees lacking water and water-logged trees), with incompatible chemicals, when ambient shade temperatures exceed 35°C, or when relative humidity is above 90% and when or when sprays are likely to take longer than 1 to 2 hours to dry. Early mornings on fine clear days are the best time to spray during hot weather. Application of higher volumes of spray to achieve greater efficacy is more effective, less risky and more cost effective than applying lower volume at higher concentrations (> 0.5% v/v). In subtropical and tropical Asia, the use of multiple, low-concentration sprays (0.25% to 0.5%) is recommended and the total concentrations applied annually should not exceed 2% in the inland provinces of China and 3.5% to 4% in the coastal provinces of China and elsewhere in Southeast Asia. Application of oil sprays should be avoided within 3 months of harvest in regions where fruit colour.

Maximum efficacy of most pesticides applied as sprays requires thorough application, irrespective of the chemical used. Such coverage is uncommon in Asia and is possibly the major reason for poor control of pests and diseases. However, even thorough coverage will not prevent establishment of HLB in citrus orchards—it only delays the inevitable. Sustainable production should be based on acceptance that trees will inevitably decline and die. Production should focus on low pesticide inputs and maintaining yields per hectare through ‘high density’ plantings, regular removal of diseased limbs and trees and replanting when necessary (‘rotation’ cropping). It should not focus on extending the life of trees beyond the point of maximum yield and on high pesticide inputs.

The impact of guava on *D. citri* and HLB, as noted above for our ACIAR project site in southern Việt Nam, appears to be real, but no replicated experiments have been undertaken. However, the impact at the site is supported by observations in orchards with and without guava interplants in the Cai Be (106° 2' E, 10° 20' N, 1 m asl) region of the Mekong Delta, and by two un-replicated experiments in Cai Be (Le Quoc Dien, SOFRI, pers. comm.). In addition to reported effects on the vector and the disease, it seems guava interplants also lead to lower populations of aphids, scales, mealybugs and citrus leafminer (Do Hong Tuan et al., SOFRI, pers. comm.), but higher incidences of citrus red mite, citrus canker (*Xanthomonas campestris* pv. *citri* (Hasse) syn. *X. axonopodis* pv. *citri* (Hasse) Vauterin, Hoste, Kersters & Swings [Pseudomonadales: Pseudomonadaceae]) and citrus scab (*Elsinoe fawcettii* Bitancourt & Jenkins [Dothideales: Elsinoaceae]) occur in interplanted orchards (Do Hong Tuan et al., SOFRI, pers. comm.). A marginal increase in the use of copper-based fungicides may be required for control of citrus canker and citrus scab in such situations. However, farmer observations suggests that interplanting with guava, regular monitoring and removal of flush growth on which psyllids are observed, application of mineral oils to flush growth, and spot-spraying with oil on other occasions, is a cost-effective management program in the Mekong Delta for controlling the psyllid and for delaying significant impacts of HLB for 15 or more years (Le Quoc Dien, SOFRI, pers. comm.). Our impression is that current interest and research on guava interplants stems from farmer observations over the past 15-20 years. We have initiated experiments with guava interplants in Indonesia and encouraged colleagues in Sarawak and China to also conduct experiments. We are interested in confirming the observations in Việt Nam and evaluating the use of guava in hilly and less humid environments than in the flat humid environment of the Mekong Delta. At this point, we assume the guava interplants at Cai Be reduce numbers of adult psyllids landing on adjoining citrus trees by interfering with one or more of the following: (a) visual recognition of host foliage, (b) masking of host volatiles, or (c) avoidance of guava volatiles. Based on current knowledge of the chemical ecology of aphids and psyllids (Kristoffersen 2003), masking and avoidance would seem to be more likely than impacts on visual cues. The observations at Cai Be also suggest that adult *D. citri*

choose to avoid landing within interplanted orchards. If proven, the use of guava interplants to control HLB and *D. citri* could have major ramifications for the citrus industries of Southeast Asia. It has the potential to provide farmers with income from guava fruit in the 3-4 years after planting before citrus trees bear, while simultaneously prolonging the viability of citrus orchards and significantly reducing expenditure on pesticides.

Nutrition. The external symptoms caused by HLB are quite variable and differ with species and variety and also for the same scion grafted onto different rootstocks. These symptoms may be affected by cultural practices such as different irrigation and fertiliser practices. The symptoms resulting from infection of existing shoots will differ from those on shoots that develop from infected tissues and symptom expression will alter as tissues age and with time after infection. Due to sensitivity to temperature of the causal organism, symptoms will vary with climate, between years and from season to season. It is also likely that symptoms may vary due to strain variation within the pathogen.

The most conspicuous symptom of the disease is a yellow shoot, as the name huanglongbing implies. In leaves, the pathogen typically causes chlorosis that resembles iron or zinc deficiency. Work in South Africa (Koen & Langenegger 1970) has shown that infection with *Ca. L. africanus* causes mineral imbalances in which potassium concentrations are raised, whilst those for calcium, iron, magnesium and zinc are reduced. These results explain the potential confusion of the symptoms of HLB with mineral deficiencies. Our work with *Ca. L. asiaticus* has shown that infected, field grown plants have reduced concentrations of iron and zinc (Arlyna Budi Pustika, UGM, pers. comm.). Young plants in pot trials also had reduced levels of iron: zinc concentrations appeared to be lower, but were not statistically different between infected and healthy plants and it may be that zinc levels are affected later than iron. Our work on the effects of infection with HLB on mineral nutrition is continuing. In addition, we are studying the effects of foliar applied minerals on disease development. We want to determine whether applications of magnesium, iron, zinc or a mix of several minerals can reduce symptom expression and extend tree life or whether applied minerals promote bacterial growth thereby increasing disease severity.

Systematics of the genus *Citrus* and *Citrus* relatives

The status of both *M. exotica* and *M. paniculata* is only one of several systematic issues of importance to effective management of HLB and its vectors. The most widely used taxonomic systems for classifying citrus are those of Walter Swingle (Swingle 1946; Swingle & Reece 1967) and Tyozauro Tanaka (Tanaka 1977). They recognised 16 and 162 species respectively. Their now dated and divergent views have led to widespread confusion in the use of names of cultivar groups, inappropriate species status of hybrids, and the names of true species (Scora 1975; Mabberley 1997), and a profound misunderstanding of generic limits (Mabberley 1998). This confusion has been exacerbated by the fact that the literature on the cultivated (and therefore naturalised) plants is in turmoil because of a plethora of species names used for apomictic hybrid clones. Furthermore, there has been no consensus on the names of these entities and many dubious synonyms and invalid names are widely used in books, journals and, most recently, in poorly referenced and inconsistent popular and technical internet websites that could perpetuate errors *ad infinitum*. Moreover, some papers, including molecular studies, deal with plants for which claimed taxonomic relationships are invalid or poorly understood and for which verifiable voucher specimens have not been preserved. Many such publications are therefore of limited value and may mislead the unwary. Recent work suggests that the genus *Citrus* comprises about 25 species (Mabberley 2004). This view is based on (i) recent reunification of *Eremocitrus*, *Fortunella*, *Microcitrus* and *Poncirus* with *Citrus* (Mabberley 1998), (ii) molecular studies by Guerra et al. (2000) and Samuel et al. (2001) that support these reunifications, and (iii) other molecular studies that suggest that the species of *Oxanthera* from New Caledonia (Stone 1985b) and *Clymenia polyandra* (Tanaka) Swingle from New Ireland in eastern Papua New Guinea should be reunited with *Citrus* (Bayer et al. 2004; Mabberley 2004). Stone (1985b) described a second species of *Clymenia*, *Cly. platypoda* B.C. Stone, from New Britain and Manus Island in Papua New Guinea, but mentioned the possibility that it could be a hybrid between *Citrus* and *Cly. polyandra*, a species considered by Swingle & Reece (1967) to monospecific.

Murraya (*sensu stricto*) was recently transferred from the Clauseneae to the Aurantieae and curry bush is once again *Berbera koenigii* L., not *M. koenigii* (L.) (Samuel et al. 2001). The currently monospecific *Merrillia caloxylon* (Ridley) Swingle may be a species of *Murraya* (Samuel et al. 2001),

and *Bergera* may be junior synonym of *Clausena*. The most recent review of *Clausena* by Molino (1994) led to circumscription of 15 species through extensive synonymy, and a conclusion that *Cl. anisata* (Willd.) Hook. f. ex Benth. is native to Asia, not Africa.

Hosts and origins of citrus psyllids

Modern psyllids, including the Diaphorininae and Triozinae, probably evolved with the Sapindales in Gondwana (Hollis 1985, 1987; White & Hodgkinson 1985). *D. citri* occurs throughout Southern and Southeast Asia from Iran to the southern islands of Japan, the Philippines, through the Indonesian archipelago to Papua New Guinea in northern Australasia, in Saudi Arabia, the Mascarenes (Réunion and Mauritius), Brazil, Argentina and northern South America, the Caribbean, Florida and Texas (Halbert & Manjunath 2004; Halbert & Núñez 2004; Weinert et al. 2004; OEPP/EPPO 2005a). The Diaphorininae have an ecological preference for dry climates (Hollis 1987). Hollis (1987) suggested that *D. citri* possibly evolved in India in association with a species of *Murraya*. In 1987 the genus *Murraya*, as circumscribed by Swingle & Reece (1967), fell within the tribe Clauseneae, not the tribe Aurantieae, and it included species now placed in the genus *Bergera*, based on studies reported by Samuel et al. (2001). Although *D. citri* was first described from Taiwan (Kuwayama 1907-1908), it was first recorded as a serious pest of citrus in India (Husain & Nath 1927) and both of its primary parasitoids, the ectoparasitoid *Tamarixia radiata* (Waterston) [Hymenoptera: Eulophidae] and the endoparasitoid *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) [Hymenoptera: Encyrtidae], were first described from India (Waterston 1922; Shafee et al. 1975). Records of these parasitoids elsewhere in Asia are related to intentional introductions (Shui et al. 1988, Waterhouse 1998) or to their movement in association with spread of their host to new regions. All known hosts of *D. citri* belong to the family Rutaceae, most within the Aurantioideae: a record on jackfruit, *Artocarpus heterophyllus* Lamarck [Rosales: Moraceae], in India (Shivankar et al. 2000) is unquestionably erroneous. In most instances, the suitability of hosts 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 nature and frequency of flush growth, and local biotypes of the psyllid. Records should therefore be interpreted cautiously, more so given the likelihood that hosts have not been correctly identified.

In addition to *D. citri*, three or more psyllids have been recorded from Aurantioideae in Southeast Asia, but none of them has been reported as a vector of the disease. *Psylla murrayi* Mathur [Psyllidae], is known to feed and develop naturally on *B. koenigii*, orange, citron (*C. medica* L.), and lime (*Citrus × aurantiifolia*) (Mathur 1975, Lahiri & Biswas 1980). Two other species in this genus, *P. citricola* Yang & Li and *P. citrisuga* Yang & Li, have also been recorded from pomelo (*C. maxima*) and citron in China (Yang & Li 1984), but Burckhardt (1994) suggested that they may be closely related to, or synonyms of, *P. murrayi*. In addition to *D. citri*, two other species of *Diaphorina* have been recorded on Aurantioideae in India: *D. communis* Mathur from *B. koenigii*, *M. paniculata* and occasionally from *Citrus* (Mathur 1975), and *D. murrayi* Kandasamy from *M. exotica*. Given that variation within widely distributed species of *Diaphorina* can be considerable between distant populations, *D. communis* and other Rutaceae-feeding congeners from India may be synonymous (see Burckhardt 1984, 1994, Halbert & Manjunath 2004). None of these psyllid species, other than *D. citri*, appear to have been recorded from species of *Clausena*. There are no records of *D. communis* or *D. murrayi* as vectors of HLB.

T. erytrae is native to sub-Saharan Africa and occurs there and in Madagascar, Mauritius, Réunion, Saint Helena, Saudi Arabia (where *D. citri* also occurs), Yemen, Portugal (including Porto Santo Island), and in the Canary Islands (Spain) (Hollis 1984; OEPP/EPPO 2005b), and is the only species of *Trioza* that is known to feed and develop on Rutaceae (Hollis 1984; Aubert 1987). *T. erytrae* has two native rutaceous hosts in Africa on which it can complete its development, *Vepris lanceolata* (Lam.) G. Don [Toddalioideae: Toddalieae] and *Zanthoxylum capense* (Thunb.) Harv. [Rutoideae: Zanthoxyleae] (Moran 1968; Hollis 1984; Aubert 1987) The psyllid also feeds on the native monospecific *Calodendrum capense* (L. f.) Thunb. [Rutoideae: Diosmeae], but cannot complete its life cycle on this species (Moran 1968). Synonyms of *V. lanceolata* include *Boscia undulata* Thunb., *Toddalia lanceolata* Lam, *Vepris querimbensis* Klotzsch and *Vepris undulata* (Thunb.) Verdoorn & CA Smith (see Mziray 1992). (*Z. capense* is often cited as *Fagara capensis* Thunb., and

sometimes as *F. capense*.) Non-native African hosts on which *T. erythrae* can complete its development in Africa include *Citrus* species and hybrids, *M. paniculata* and the *Cl. anisata* (= *Cl. inaequalis* (DC.) Benth.) (Moran 1968; Hollis 1984; Aubert 1987). The latter species is often stated as being native to Africa (e.g., Moran 1968; Aubert 1987; OEPP/EPPO 2005b) and is host of the *Ca. L. africanus* (Korsten et al. 1996). It is, however, native to the Western Ghats of India and the northeast part of the Indian subcontinent through to China and has a plethora of synonyms too long to list here (Molino 1994). Although *Cl. anisata* is the second-most favoured non-citrus host of *T. erythrae* in Africa (Moran 1968) there does not appear to be, as there does for *Cl. lansium* (Xu et al. 1988), which is also a host for *Ca. L. asiaticus* (Ding et al. 2005), any report of *D. citri* feeding or developing on it in Asia. The fact that *V. lanceolata* is the preferred native host of *T. erythrae* in Africa (Moran 1968) suggests that it is be the original host of this psyllid. In the context of the following discussion on plate tectonics and the origins of *Citrus* it is of interest to note that no psyllids have been recorded on *Citrus* or *Murraya* in Australasia (Paul De Barro, CSIRO, pers. comm.). Furthermore, the liberibacters that cause HLB do not occur naturally in Australasia.

On the origins of *Citrus*

Scant attention has been given by horticulturists, pathologists and entomologists to the biogeography of *Citrus* and its close relatives since the impact of plate tectonics on the geography of the Earth were accepted in the 1960s and 1970s. The current perceptions of Southeast Asian origins for *Citrus* is based on Swingle's narrow circumscription of the genus, as prepared in 1946, and widespread ignorance of the origins of both Southeast Asia and Australasia and how the landmasses are related. In reviewing the likely geological origins of *Citrus*, we initially considered the possibility that the genus evolved in Eastern Gondwana some 100 million years ago (mya) before the then extant components of Gondwana separated to gradually form South America, Africa, Madagascar, the Arabian Peninsular, the Indian subcontinent, Antarctica and Australasia. We now consider this unlikely, as there are no known extinct or extant species of *Citrus* that are native to Africa or Madagascar and the north-eastern region of the Indian subcontinent appears to represent the western-most limit to the distribution of true citrus species. The eastern-most limit to the distribution of true species is New Caledonia, which has been in the same relative position, some 1200 km east of Australia, for 55 mya: about the time that India collided with Asia (Hartley 2001a). The flora of New Caledonia contains many groups of plants that appear to be remnants of the late Cretaceous-early Tertiary (97 to 23 mya) Gondwanan flora that once covered large parts of Australasia. As New Caledonia separated from Australia and drifted to its present position, it carried with it a sample of this early flora. The descendants of these plants have been able to survive in the island's relatively stable climate, while most of their relatives in Australia and other parts of the region were lost due to climatic changes (especially drying) that took place in Neogene times, 23 to 1.6 mya (Lowry 1996). Did the original Australasian species of *Citrus* follow a similar evolutionary path?

In commenting on the origins of *Microcitrus*, which they considered a primitive genus related to *Citrus*, Swingle & Reece (1967) opined that 'these remarkable citrus fruits are extremely interesting, in that they show how evolution has proceeded in regions isolated as Australia and New Guinea have been during the last 20 or 30 my since they were cut off from all other landmasses. Swingle & Reece 1967 also stated 'perhaps some of its species are very like the ancestral species from which *Citrus* developed' and 'the evolution of other citrus fruits is not so easily followed, since *Citrus*, *Fortunella*, and *Poncirus* did not originate in regions that were geographically isolated in definitely dated geologic eras.' They quoted Brough (1933), who in turn quoted Benson (1923), to support his view that 'Australia is considered to have been joined to the Asiatic mainland at least during the Cretaceous period, but probably a complete separation has existed since the beginning of the Eocene.' Swingle & Reece (1967) concluded that 'The migrations of higher plants into Australia are held to have occurred during later Cretaceous times.' The notion of the distribution of the Aurantioideae from Southeast Asia through Malesia to Australasia during the Neogene or Quaternary (up to 1.6 mya) periods, more recently than the Cretaceous era (146 to 65 mya), was considered likely as recently as the 1990s (Stace et al. 1993) in relation to movement of plants across 'land-bridges' (Armstrong 1975; Barlow 1981). Australia has never been joined to the Asiatic mainland (Hall 1997, 2001, 2002; van Welzen et al. 2005) and there is no record of species of *Citrus* being native to Wallacea, the islands between

Wallace's Line in the west and Lydekker's Line, which runs along the Australasian continental shelf in the east.

Swingle's view was that the genus *Citrus* may have originated in the New Guinea-Melanesia region, and that it evolved into fragrant, delicious-flavoured species from a few species with sour and bitter-flavoured, almost inedible fruit, such as *C. hystrix* and *C. ichangensis* Swingle, that had developed in East Indian Archipelago, in the Philippines, New Guinea and Melanesia. He regarded this evolutionary path as the culmination of a very long period of progressive evolution that certainly began before Australia was cut off from land connection with New Guinea and Asia, probably more than 20 mya. He regarded the genera *Eremocitrus*, *Fortunella*, *Microcitrus* and *Poncirus* as ancestral to his circumscription of *Citrus*. He regarded *Cly. polyandra* as monospecific and probably the most primitive of all the genera within the 'True Citrus Fruit Trees', and with an extraordinarily close resemblance of leaves and petioles of those of *Monanthocitrus*, represented by the then monospecific *Monanthocitrus cornuta* (Lauterb.) Tanaka (there are now four described species of *Monanthocitrus*: see Stone & Jones (1988)). This convinced Swingle that *Cly. polyandra* was an entirely new type of citrus fruit tree, possibly having descended from a remote ancestral species common also to *Monanthocitrus*. He considered *Oxanthera*, which has large, white fragrant flowers very much like those of *Citrus*, to be a highly specialised xerophytic genus that possibly developed from the common ancestor of the genera *Wenzelia* and *Monanthocitrus*, and that the latter arose from an ancestral form much like that of some species of *Wenzelia*. He placed *Oxanthera*, *Wenzelia* and *Monanthocitrus* with several other 'minor citroid' genera in the subtribe Triphasiinae. It is now clear that the species of *Oxanthera*, as species of *Citrus* (see Bayer et al. 2004), belong to the true citrus group of fruit trees within the Swingle's subtribe Citrinae.

Most of the landmass Southeast Asia is derived from a complex agglomeration of terranes now far removed from their Gondwanan origins. North and South China (excluding Hainan and Taiwan), Indochina, Myanmar (Burma), Thailand, Malaysia and western Indonesia rifted from northeast Gondwana in three significant tectonic events starting in the Early Devonian (about 400 mya) and ending before the origin of Angiosperms (flowering plants) about 145 mya in the Early Cretaceous in the African component of Gondwana (Raven & Axelrod 1974; Burrett et al. 1991; Metcalfe 1991, 1998; Scotese 1991; Crane et al. 1995; Morley 1998; Baillie et al. 2004; Burgoyne et al. 2005). The Rutaceae appeared in Africa-South America in the Mid Cretaceous, about 90 mya (Raven & Axelrod 1974).

Australasia comprises Australia (including Tasmania), New Guinea, the Bismarck Archipelago Solomon Islands, New Hebrides, Fiji, Lau, Tonga, Kermadec Islands, New Caledonia New Zealand. Progressive separation of the now western margins of this region from India from 96 mya in the Late Cretaceous to 35.5 mya in the Late Eocene marked the second major phase in the breakup of eastern Gondwana. Separation of Madagascar from India, Australia from Antarctica, and New Zealand and New Caledonia from Australia also began about 96 mya in the Late Cretaceous. Separation of Australia and Antarctica ended when sea formed between the Tasmania (then still linked to the Australian landmass) and Antarctica about 35.5 mya (Hartley 2001a, b). India rifted northwards to collide with Asia in the Middle Eocene about 50 to 55 mya. At this point, both the northern margin of the Indian plate and Sundaland (Java, Sumatra, Borneo, western Celebes, Malaya, Shan and peninsular Myanmar, Thailand and Indochina) experienced an ever-wet, equatorial climate (Morley 1998).

Southern New Guinea is part of the Australasian craton whereas the northern sector comprises accretions of terranes of Pacific and Gondwanan origins, the latter including terranes that rifted into the Pacific and then back (e.g. New Britain and New Ireland). The terranes that form Wallacea originated from parts of Australasia, Gondwanic and Pacific accretions, and volcanoes following the collision of Australasia with Sundaland about 20 mya in the Middle Miocene (16 to 11.6 mya). These events over the past 55 million years have been most recently summarised by several authors (Veevers et al. 1991; Metcalfe 1998; Hall 1997, 2001, 2002; Hartley 2001a, b). Animations of events over the past 55 my can be viewed on the internet (SE Asia Research Group 2006).

These animations and detailed documentation of reconstructions (Hall 2001a, 2002) suggest that favourable opportunities existed for migration of *Citrus* from eastern Australasia to Asia via island arcs in the Pacific Ocean from the Bartonian (40.4 to 37.2 mya) stage of the Middle Eocene epoch (48.6 to 37.2 mya) until the present age, and likewise for migration from Asia to Australasia. Most dispersal could have been endozoochorous, through migrations of birds and bats. Evidence to support

this is scant, as there have been no detailed studies. Mature fruits of extant native Australasian *Citrus* are relatively small compared to most extant cultivated Asiatic forms. Mature fruit of native Australian rainforest species are green. This suggests that their seeds may be dispersed by palaeotropical, non-echolocating, frugivorous, pteropodid bats; birds are more closely linked to dispersal of brightly coloured red and orange fruit (Hodgkison et al. 2003; Ingle 2003). However, mature fruits of extant citrus relatives native to New Guinea range from green to orange and red: *Mo. cornuta* fruit are small and red, and those of *Wenzelia dolichophylla* (Lauterb. & K. Schum.) Tanaka are red (Swingle & Reece 1967). Such endozoochorous dispersal would have been aided by formation, movement and accretion of terranes to the east, north and west of New Guinea over the past 37 my (see Burrett et al. 1991; Hall, 2001a, 2002), particularly terranes that now form the Halmahera Islands and most of the Philippines (see Hall 2001a, b; Morley 2003). Evidence to support possible endozoochorous dispersal of primitive *Citrus* germplasm is provided by Hartley (2001a) in his treatise on the taxonomy, origins and biogeography of the Australasian genera *Euodia* and *Melicope*.

In light of the information presented above it would seem probable that species of *Citrus* in Australasia could be more primitive than conspecific species in Asia, and that *Citrus* evolved in Australasia. However, recent molecular studies (Bayer et al., Australian National Herbarium) suggest that the Australasian species are younger than those in Asia. These molecular studies strongly support Asiatic origins of the genus, but the impact of climate change on extinction of species and the evolution of others as Australasia moved northwards and fragmented, and relationships of *Citrus* to ancestral genera need to be resolved before a conclusion can be reached. In any case, the evidence we present in this paper suggests that HLB did not evolve with *Citrus* in Southeast Asia or Australasia. In the next two sections we discuss possible origins of the pathogen(s) in relation to the movement of *Citrus*.

Movement of cultivated *Citrus* to Europe and Africa

Movement of plants by humans frequently leads to the transfer of pests and diseases from native to non-native regions. Such movement has played a major role in the spread of HLB (Halbert & Manjunath 2004). Movement of cultivated forms of *Citrus* from Asia to Europe first occurred about 327 BC, when Alexander the Great took the citron, probably of Indian origin, to the Mediterranean from Persia (Ramón-Laca 2003; Mabberley 2004). This was followed by the sour orange, the lemon, the lime, and the pomelo, all from Indochina about 900 through Muslims settling and living on the Iberian Peninsula and in Sicily. The sweet orange and the mandarin were introduced, presumably from China, relatively recently, about 1400 and between 1700 and 1800, respectively, as a result of trade with the British and Portuguese colonies (Ramón-Laca 2003). Oranges derive from crosses between the mandarin and the pomelo, the first from China and the second probably from Indochina. The geographical isolation of these progenitor species was probably broken down by humans, such that when they met, spontaneous hybrids formed, with apomictic clones being selected for propagation. The lemon is an orange × citron hybrid that presumably originated in the Indian subcontinent. One genome of the lime (*C. × aurantiifolia*) is the pomelo, the other unknown, but is possibly *C. inchangensis*, a plant said to be allied to *C. hystrix*, and a plant widespread in Southeast Asia (Mabberley 2004). The citron was introduced to China from India around 700 (Mabberley 2004). Introductions of cultivated citrus to southern Africa, the Americas and Australasia followed European invasions of these regions after 1700.

The introduction of *Citrus* to Africa may also have occurred several centuries before European colonisation, possibly as far back as 500 AD from the Malay Archipelago and China given the extent of trade between and within these regions over the past 2000 years (Tolkowsky 1938; Levathes 1994; Munoz 2006). Portuguese discoverers of the all-sea route from Europe to India in the 1400s found oranges, lemons and citrons on the east African mainland between modern Kenya and Mozambique and on the islands of Zanzibar. In the 1400s, ships could sail between India and Madagascar in 20 to 25 days, thus permitting conveyance of citrus fruits, seeds, cuttings and even young trees but cultivation of citrus as far south as the Cape of Good Hope did not occur until as late as the 1650s when fruits, including oranges, were introduced by the Dutch (Tolkowsky 1938).

The disease and its origins

As noted in our introduction, the origin of HLB is currently considered to be China. This assumption is based on the 'first' record of symptoms of the disease being made in southern China in the late 1800s (Lin 1956; Reinking 1919; Zhao 1981; da Graça 1991), and presumably to perceived origins of *Citrus* and an assumption that the disease evolved with *Citrus*. Early Indian records of symptoms resembling those of the disease seem to have been ignored. These records, which can be interpreted as being at least as descriptive as those of Reinking (1919), suggest symptoms resembling those of HLB in *Citrus* were observed in the mid 1700s in the central provinces of India by Roghoji, the Bhonsla Raja of Nagpur. Nagpur is now the 'orange' capital of modern Maharashtra. Subsequent, relatively early records in north-western and north-eastern India occurred in the 1800s and early 1900s (Bonavia 1888; Husain & Nath 1927; Pruthi & Mani 1945; Asana 1958; Capoor 1963; Fraser et al. 1966, Chadra et al. 1970; da Graça 1991). With the exception of China, such symptoms were not recorded in Southeast Asia until after 1940: in Malaysia in the 1970s (Ko 1988), in the Philippines in 1957 (Martinez & Wallace 1967), Thailand in the 1960s (Schwarz et al. 1973), and in Indonesia in the 1940s (Aubert et al. 1985). Human-assisted spread of the disease through the Indonesia archipelago to Papua New Guinea (northern Australasia) took some 60 years: it was first recorded Papua New Guinea in the northwest at Vanimo in 2002 (Weinert et al. 2004).

This evidence suggests eastward movement of the disease and *D. citri* from the Indian Subcontinent to Southeast Asia, particularly China, in relation to movement of plants as part of sea trade (Levathes 1994; Munoz 2006). We suspect that the disease and the psyllid were introduced to China from the Indian subcontinent during the late 1700s or early 1800s. But did the disease originate in association with *Citrus* and *D. citri* in India, or elsewhere in Asia? And is there a single species of *Liberibacter* or three (*Ca. L. asiaticus*, *Ca. L. africanus* and *Ca. L. americanus*) and a subspecies (*Ca. L. africanus* subsp. *capensis*), as currently reported (da Graça & Korsten 2004; Teixeira et al. 2005a, b)? Our opinions differ about the validity of the three species of *Liberibacter*, but we believe their status as separate species needs to be defended and to take into account (a) the probability of three species evolving in an initial association with what we assume was a single, most probably asymptomatic, host plant species and a single psyllid vector, and (b) the probabilities of the three species evolving on three continents in association with different host plants and the two known vectors of the disease, after the separation of Gondawana. The issues go beyond the accuracy of current methods for determining species of fastidious phloem limited bacteria: if the current species represent forms of a single species that has adapted to new hosts and environments in the recent past (perhaps < 500 years) such adaptations are likely to continue. This could thwart use of 'resistant' plants and the development and success of biosecurity measures. If the genus is monotypic, and if a distant relative of *Citrus* is the original host, then the range of susceptible species may be quite broad, and not readily predictable, rather than relatively narrow. Bastianel et al. (2005) demonstrated that within a given geographical region, *Ca. Liberibacter asiaticus* may comprise several different variants. Halbert & Manjunath (2004) proposed strain variation in the pathogen to explain consistent detection of HLB in *M. paniculata* by Tirtawidjaja (1981). However, current studies (see above) at UGM suggest that the plants in which symptoms were observed may have been *M. exotica*.

We hypothesise that the disease originated in Africa. Africa is the only region in which a common association between the disease and a seemingly asymptomatic and preferred host of a vector appears to occur: we think the disease may have originated in sub-Saharan Africa in an association involving *T. erythrae* and *V. lanceolata*. We consider it highly unlikely that the disease originated in Asia in association with *Citrus* or any *Citrus* relative in which severe symptoms, including death, occur. There would be a high probability that any such association would lead to rapid extinction of the host plant(s). As noted above, records indicate that the disease has spread slowly, from west to east, through Southeast Asia over the past 250 years. There is no indication that the disease evolved with native *Citrus* species in Asia or Australasia or China. There is no logical reason, given the paucity of native species of Rutaceae and psyllids in South America, to support notions that the disease, in any form, evolved in South America. Moran (1968) suggested that *V. lanceolata*, *Cl. anisata* and *Z. capense* could be the original native hosts of *T. erythrae* in Africa but, as noted above, *Cl. anisata* is not native to Africa and *Z. capense* is a relatively poor host. da Graça (1991) mentioned that efforts (JV da Graça & SP van Vuuren unpublished data) to transmit the African form of the disease to *V. lanceolata*, *Cl. anisata* and *Z. capense* by *T. erythrae* were unsuccessful, but he did not mention how the tests were done, or whether there was an expectation that the plants would exhibit symptoms of the

disease as normally observed on citrus. However, subsequent studies indicate that *Cl. anisata* and *V. lanceolata* can harbour the bacterium. van den Berg et al. (1991-1992) reported detection of the disease from psyllid infested *Cl. anisata* growing in close proximity to a HLB-infected citrus orchard. Detection was based on tests involving grafting of sweet orange indicator plants to the *Cl. anisata* trees. Simultaneous tests with the native *V. lanceolata* and *Z. capense* were negative (van den Berg et al. 1991-1992), but Korsten et al. (1996), using dot blot hybridisation, detected the bacterium in a leaf sample taken from a *V. lanceolata* tree on which many *T. erytrae*-induced leaf deformations were observed. van den Berg et al. (1991-1992) recommended removal of *Cl. anisata* plants growing near citrus orchards and Korsten et al. (1996) concluded that *V. lanceolata* is a reservoir plant for the pathogen. Neither van den Berg et al. (1991-1992) nor Korsten et al. (1996) mention the presence of HLB symptoms in any of these non-*Citrus* hosts of *T. erytrae*.

Based on this evidence and movement of plants by humans we consider it plausible that HLB was transmitted from *V. lanceolata* to orange or mandarin trees by *T. erytrae* in one of the European colonies on the southeast coast of Africa and then taken to the Indian subcontinent in infected plants or budwood some 300-500 years ago. It was then acquired and spread by *D. citri*. Spread would also have occurred through marcotting and grafting, and enhanced by changes in horticultural practices that through increased use of irrigation and fertilisers within monocultures would have led to more abundant and frequent growth flushes. The latter would have led to far higher populations of *D. citri* than would have occurred in its original environment.

Germplasm conservation

The spread of HLB eastward through Asia to New Guinea (see above) poses a major threat, not only to commercial production of citrus in Australasia, but also to generally rare native non-commercial species of *Citrus* and other Rutaceae in the region. Steps need to be taken to protect these species from possible extinction. Species we consider to be most threatened are *C. polyandra* (= *Cl. polyandra*), *C. warburgiana* F. M. Bailey and *C. wintersii* Mabberley. The first of these species is native to New Ireland and the other two to mainland New Guinea. We are also concerned about species of *Wenzelia* and *Monanthocitrus* in New Guinea and Borneo: see Swingle & Reece (1967), Stone (1985b) and Stone & Jones (1988) for the species and their distributions. Should the disease and one of its vectors become established in Australia the six Australian species of *Citrus* will be threatened: *C. australis* (Mudie) Planchon, *C. australasica* F. Mueller, *C. garrawayi* F. M. Bailey, *C. glauca* (Lindley) Burkill, *C. gracilis* Mabb., *C. inodora* F. M. Bailey. Likewise, despite their relative isolation, the 5-6 species of *Citrus* (*Oxanthera*) in New Caledonia are at risk. *C. australasica* was recorded by Aubert (1987, 1988) as a common host of *D. citri* in Réunion and the psyllid completed its development on this host. *C. australasica* and *C. australis*, *C. wintersii* and *C. × virgata* have been recorded common hosts of *D. citri* in Florida (Halbert & Manjunath 2004). Aubert (1988) also cites *C. australasica* as an occasional host of *T. erytrae* on which this African psyllid fed but did not oviposit. There is no evidence in the literature to suggest that any Australasian species of *Citrus* will not be susceptible to the disease, but their suitability as hosts of the vectors will be influenced by the environments in which they occur and their suitability for development of the vectors. We are seeking avenues to protect all of these species and others in Southeast Asia, including the *C. halimii* BC Stone. This comparatively rare plant, which attains heights of up to 21 m, occurs in forests at altitudes ranging from 275 m to 1,830 m asl in Thailand and Malaysia (Stone et al. 1973; Stone & Jones 1988). These altitudes are below those at which *D. citri* occur in the region, and characteristic zinc deficiency or HLB mottling of leaves of one small tree in the botanic gardens at the University of Malaya were observed by two of the authors (GACB & DJM) in April 2003.

Incursion management

Pre- and post-incursion management plans are being developed through funding by Horticulture Australia Limited to protect the Australian citrus industry and native species of *Citrus* from HLB. Distributions of known and potential hosts of the pathogens and their vectors are being determined. Surveys have also determined the absence of native psyllid species feeding or developing on Australian Aurantioideae (Paul De Barro, CSIRO, pers. comm.). Strict quarantine measures are in place and the Northern Australia Quarantine Service monitors known hosts of HLB and the vectors in coastal areas of northern Australia. The greatest threat is from the spread of the *Ca. L. asiaticus* and

D. citri though New Guinea towards the Torres Straits and Cape York in Northern Queensland and subsequent movement of infected and infested plant material and wind dispersal of infective adult psyllid females. Wind dispersal is also possible, though less likely, from the Indonesian archipelago. Strategies are being developed to address separate incursions by the disease or its vectors, or incursions involving the disease and a vector. The dispersed nature of the Australian citrus industry will favour containment and eradication, but the widespread distribution of *M. paniculata*, a common plant in many home gardens in which oranges, mandarins, limes and lemons also grown, may hinder eradication programs. The need to conserve native *Citrus* species in eastern Australia is being considered. An incursion of HLB in the absence of its vectors will most probably lead to the death of infected host plants and, therefore, is not a severe threat to the industry. Incursions of one or both known vectors of the pathogen will lead initially to eradication programs and should these fail, to IPM programs based on introduced parasitoids, native predators and, where feasible, biorational pesticides. Our observations in Asia suggest that both known vectors will survive in all commercial citrus regions in Australia. These observations suggest that high summer temperatures, which occasionally exceed 40°C, will not limit the distribution of *D. citri* in Australia; nor will they be high enough to limit the distribution of *Ca. L. asiaticus* in tree canopies, and therefore numbers of infective psyllids, as we suspect occurs in Pakistan from mid spring until late autumn. Based on information about the distribution of *D. citri* in China (Yang et al. 2006), it is unlikely that the lowest temperatures recorded in the citrus growing regions of Australia will prevent establishment of the psyllid in these regions. The greatest threat to the Australian citrus industry will be from a simultaneous incursion of both *Ca. L. asiaticus* and *D. citri* and significant spread by both before detection. The seasonal incidence of the less heat tolerant *Ca. L. africanus* in South Africa supports this hypothesis (Bové et al. 1974).

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