Asian Form of Huanglongbing

Subjects: Agriculture, Dairy & Animal Science

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, transmits '*Candidatus* Liberibacter asiaticus' (*C*Las), a phloem-limited bacterium associated with the severe Asian form of huanglongbing (HLB), and the most destructive disease of citrus. The pathogen and the psyllid, both of South Asian origin, are now widespread in citrus regions of Asia and the Americas. There is no cure for the disease. Application of synthetic pesticides, in some instances more frequently than fortnightly, to minimize incidence of ACP in citrus orchards, has not prevented inevitable impacts of the disease in regions of Asia where *C*Las is present. Despite the inevitable spread of the disease, significant progress has been made in Sarawak since the mid-1990s towards effectively implementing integrated pest management (IPM) programs for stemming the impact of the disease and detrimental consequences of over-reliance on synthetic pesticides. Growers are encouraged to plant pathogen-free trees, remove diseased trees, monitor incidence of the psyllid, and to use pesticides judiciously to reduce their detrimental impacts on natural enemies. Knowledge has been enhanced through research on seasonal incidence of the psyllid, use of mineral oils, development of protocols and iodine–starch test kits for detecting infected trees, PCR for confirming the presence of *C*Las in symptomatic leaves, methods for monitoring incidence the psyllid, and training extension staff and growers. However, major impediments to increasing the average longevity of trees beyond <5 years in poorly managed orchards, based on marcotting (air layering), and >12 years in well-managed orchards, based on pathogen-free trees, still need to be addressed. These include grower knowledge, marcotting, aggressive marketing of synthetic pesticides, high prices of mineral oils, spray application procedures, and better reliance on natural enemies of the psyllid.

citrus | Diaphorina citri | 'Candidatus Liberibacter asiaticus'

1. Introduction

The genus *Citrus* (Rutaceae: Aurantioideae: Aurantieae) includes species and hybrids of widely cultivated fruits (grapefruit, kumquats, lemons, limes, oranges, and pomelos) in tropical and subtropical regions of the world [1][2][3][4]. The genus is believed to have originated from the region comprising Northeast South Asia, South and Southwest China, Indochina, and Malesia [5]. In Malaysia, citrus is grown in commercial orchards, small holdings, and backyards. It is an important fruit crop in the East Malaysian state of Sarawak in northern Borneo, where it provides a livelihood to many growers who supply mainly to the domestic market. The total area of commercial orchards in the state was approximately 3250 ha in 2005 [6]. An estimated 28.35 metric tonnes of fresh orange fruit/juice were exported to Singapore, Peninsular Malaysia, and Brunei in 2005. The most important production areas in Sarawak are located in the Samarahan Division adjacent to the state capital Kuching (1.5548° N, 110.3594° E), with some major plantings in Sarikei, Sibu, and Bintulu Divisions, some 140, 190, and 350 km, respectively, northeast of Kuching.

The major impediments to production are the severe Asian form of huanglongbing (HLB) putatively associated with a phloem-limited Gram-negative bacterium '*Candidatus* Liberibacter asiaticus' (CLas) (α-Proteobacteria) and the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psyllidae), the most widely distributed vector of the pathogen that has severely affected citrus production in Asia and the Americas [7][8][9][10][11][12]. The psyllid was noted as being present in Malaysia by Clausen [13]. Symptoms resembling HLB were first noticed in Malaysia in the 1970s in lowland peninsular orchards near Kampung Jerangau (4.8370° N, 103.1971° E, 20 m above sea level (ASL); Kuala Dungun, in Terengganu; Ulu Tiram, in Johor (1.5974° N, 103.8150° E, 25 m ASL); and the highland area near Ringlet (4.4146° N, 101.3846° E, 1110 m ASL) in the Cameron Highlands in Pahang [14][15][16][17], but no bacterium-like organisms were observed in sectioned fixed leaves examined under an electron microscope in France [18]. Broadbent [18] observed symptoms typical of the disease in grapefruit (*Citrus* × *aurantium* L.), sweet orange (*C.* × *aurantium*), and mandarin (*C.* × *aurantium*) leaves in the Cameron Highlands. Grapefruit were severely affected while lemons (*C.* × *limon* (L.) Osbeck), limes (Mexican, Key, alemow: *C.* × *aurantiifolia* (Christm.) Swingle, syn. *C.* × *macrophylla* Wester), and Dancy tangerine (*C.* × *aurantium*) appeared to have some tolerance. However, no bacterium-like organisms were observed in sections of young leaves [18]. Symptoms were also reported from the Cameron Highlands by Catling [19]. Information provided by Ko [20] and Saamin et al. [21] suggests that the first record of the disease in Peninsular Malaysia was related to the introduction of *C.* × *aurantium* seedlings, in this instance Tanaka's '*C. suhuiensis*', a mandarin variety from Sihui (23.3534° S, 112.8964° E), near Guangzhou in Guangdong, mainland China, to Terengganu, from China in the 1950s and 1960s. The presence of the disease was confirmed when the pathogen *C*Las was detected in sectioned leaves from symptomatic plants in the Cameron Highlands in 1987 [22][23][24]. The disease was first detected in Sarawak in the Samarahan Division in 1988 [25]. Since then, research and extension activities undertaken by the Sarawak Department of Agriculture (SDOA) and universities in the state have focused on minimising the impact of the disease. Intensive pesticide programs lead to insecticide resistance and are harmful to pollinators and beneficial insects.

Therefore, it is important to design IPM strategies that minimise the utilisation of pesticides, especially neonicotinoids and other broad-spectrum materials, and maximise the use of natural enemies, especially the primary ectoparasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and the primary endoparasitoid *Diaphorencyrtus aligarhensis* (Shafee, Alam and Argarwal) (Hymenoptera: Encyrtidae), as well as several predators, including the weaver ant, *Oecophylla smaragadina* Fabricius (Hymenoptera: Formicidae).

2. Origin and Distribution of Asian HLB

Although the disease was first recognized as transmissible in China [8], the first unambiguous description of symptoms is within text in which Husain and Nath [26] describe damage caused by the psyllid: small and insipid fruit, dieback, and death of trees caused by a 'toxin' injected into plant tissues by the psyllid. The pathogen probably originated in association with a non-citrus host in South Asia. It did not originate in mainland China or Taiwan. The disease was not described, as oft cited, by Reinking [27], who observed citrus maladies in Guangdong in mainland China, or in Taiwan by Sawada (1913) [28], as claimed recently by Zhou [29] and Guo et al. [30]. The first authentic record of HLB in mainland China appears to have been made in Guangdong in 1938 [31][32], four years after ACP was recorded in the province [33][34][35]. HLB was first recorded in Taiwan in 1951 [35][36]. It is now widespread in Asia, the Americas, and is present in the Mascarene Islands, New Guinea, and, most recently, Africa [7][37][38][39][40][41][42].

2.1. Host Susceptibility to HLB

All citrus species and hybrids are susceptible to the Asian form of HLB, although some species, e.g., *C. cavaleriei* H. Lév. ex. Cavalerie (China), *C. gracilis* Mabb. (Australia), and *C. hystrix* DC (Indochina and Malesia), have not been evaluated or fully evaluated. The least susceptible species, in approximate order of increasing susceptibility, with their geographic origin in parentheses, include *C. glauca* (Lindl.) Burkill (Australia); *C. halimii* B.C. Stone (Malaysia and Thailand); *C. australasica* F. Muell., *C. inodora* F. M. Bailey, and *C.* × *virgata* Mabb. (Australia); *C. (Poncirus) trifoliata* L. (China); *C.* × *oliveri* Mabb. (*C. australasica* × *C.* × *microcarpa* Bunge) and *C. australis* (A.Cunn ex Mudie) Planchon (Australia); and *C. latipes* (Swingle) Tanaka (Northeast South Asia) [43][44]. The susceptibility to CLas of species of *Citrus* (*Oxanthera*) from New Caledonia and of *C. polyandra* Tan. (syn. *Clymenia polyandra* (Tan.) Swingle) from New Ireland in Papua New Guinea has not been determined.

In Malaysia, Shokrollah et al. [45] assessed the susceptibility of 18 citrus 'species' to HLB following graft transmission of *C*Las from infected mandarin plants. The pathogen was detected by PCR in 15 of the species 6 months after grafting. Susceptibility was categorised into five symptom groups: severe (72–58% severity), comprising 'limau madu' and Cleopatra mandarins, and sweet orange; moderate (50–41% severity), comprising kumquat (*C.* (*Fortunella*) *japonica* Thunb. cv. Kasturi Chinai), *C. aurantiifolia* (cv. 'machrophylla'), and calamondin (*C.* × *microcarpa* Bunge); mild (25–17% severity), comprising citron (*C. medica* L.), *C. aurantiifolia*, *Citrus* sp. (natural genotype), and rough lemon (*C.* × *otaitensis* (Risso and Poit.) Risso syn. *C. jambhiri* Lush.); tolerant (no symptoms but PCR positive), comprising sour orange (*C.* × *aurantium*) and *C. aurantiifolia* (cv. 'limau nipis')); and resistant (no symptoms and PCR negative), comprising pomelo (*C. maxima* (Burm.) Merr., cv. limau Bali), limau purut or leech lime (*C. hystrix*), and *Citrus* sp. (cv. 'Limau Tembikai').

*C*Las-infected trees become unproductive (30–100% reduction in yield) and may die in 5–8 years, or less, of infection, depending on the susceptibility to the disease of the host plant species/hybrid [8]. Short lifespans (<3 years) of trees are often related to marcotting (air layering) (Beattie, personal observation). There is currently no cure for HLB, the best control strategy being the prevention of the disease [8]. Movement of infected citrus material by land, sea, and air is the most efficient way of disseminating HLB over large distances. Entry of *C*Las to parts of Southeast Asia and Malesia, and to the Mascarene Islands, has been linked to human migration and movement of plants from China, after the pathogen was introduced to China [10][39][46][47]. The use of disease-free planting material from reliable sources is the first and most important step towards good disease management. Mother trees from which budwood is obtained must be kept in well-maintained insect-proof screenhouses.

Initial lack of awareness of this disease, slow introduction of diagnostic tests, and rapid propagation of non-certified planting material by citrus growers to expand their orchards have contributed to poor management of HLB in Malaysia. The reluctance of growers to remove infected trees due to limited knowledge about the pathogen, its mode of spread by ACP, and the cost of vector control, have contributed to the problem. Surveys in 2004 by Azizah and Zazali [48] revealed that approximately 70% of the cultivated area (3526 ha) of citrus in Peninsular Malaysia was affected. In Terengganu, the area affected increased from 641 ha in 2001 to 1262 ha in 2004 [48].

HLB in Sarawak destroyed 1143 ha of orchards, comprising 'limau langkat' (*C.* × *aurantium*, Tanaka's '*C. suhuiensis*') or mandarin orange, sour or bitter orange, and leech lime, in the Samarahan Division by 1991. Economic losses in the state were estimated to be 6.5 million Malaysian ringgit (RM) (1.6 million United States dollar (USD) [49][50][51]. Citrus fruits had to be imported from neighbouring countries, such as Indonesia and Thailand, to meet the local demand. After the decline of the

citrus industry in the Samarahan Division, the SDOA started producing disease-free planting material in 1996. The disease-free planting material was produced using budwood obtained through shoot tip micro-propagation [52].

3. Origins and Distribution of ACP

ACP originated on the Indian subcontinent. Spread of the psyllid eastward to Southeast and East Asia appears to have been linked to movement of infested host plants from Southern India and Sri Lanka to Java in Western Indonesia, and subsequently to Ambon and Timor in Eastern Indonesia, Luzon in the Philippines, Macao, and Taiwan [46]. Although ACP was first described from specimens collected from citrus in 'Shinchiku Prefecture' in northern Taiwan [53], then Japanese occupied 'Formosa', it was first recorded in Java in 1900 [46]. The psyllid now occurs in South Asia, East Asia, Malesia, New Guinea in Australasia, Arabia and Israel in West Asia, Caribbean islands; North, Central and South America; some Pacific Ocean islands; and the Maldives and Mascarene islands in the Indian Ocean [46]. It was recorded in Africa in 2015 [54] and was recently recorded in Israel in 2021 [55].

3.1. Host Susceptibility to ACP

Known hosts of ACP were reviewed by Beattie [56]. Among 87 seed-source field-planted seedlings of 76 *Citrus* species and hybrids, and 11 *Citrus* relatives colonised by ACP adults over 4 months in summer and early autumn in Florida, the least susceptible *Citrus* species and hybrids were *C. trifoliata*, *C. inodora*, *C. glauca*, *C. halimii*, *C. australasica*, *C.* × *virgata*, *C.* × *leiocarpa* hort. ex Tan., *incertae sedis* (Koji mandarin), and sour orange. The most colonised, including *Citrus* relatives, were *C. reticulata*, *Bergera koenigii* L. (curry leaf) (Aurantioideae: Clauseneae), *Murraya paniculata* (L.) Jack (orange jasmine) (Aurantieae), *C. medica*, and *C. aurantiifolia* (lime, Key or Mexican lime, alemow) [57]. Lim et al. (1990-citrus greening) [24] cited *C. hystrix*, *C. maxima*, *C. reticulata* L. (including *C. suhuiensis*), *C.* × *aurantiifolia*, *C.* × *limon*, *C.* × *microcarpa* Bunge, Rangpur lime (*C.* × *otaitensis*), *B. koenigii* and *Clausena excavata* Burm.f. (Clauseneae), and *M. paniculata* as hosts in Malaysia. In Sarawak, Leong et al. [57] reported that ACP can colonise and breed on 'limau langkat', orange jasmine, and curry leaf, and that orange jasmine is the preferred host. ACP completes its life cycle (from egg to adult) in the shortest period (18.5 days) on orange jasmine, followed by 'limau lankat' (19.0 days) and curry leaf (23.0 days) [57]. The susceptibility of species of *Citrus* (*Oxanthera*) from New Caledonia and *C. polyandra* from Papua New Guinea as hosts of ACP has not been determined.

3.2. Biology of ACP and Influence of Abiotic Factors

ACP has a high capacity to increase in numbers on highly suitable hosts in monocultures [26][58][59], but in the absence of HLB it is a minor pest. It is not able to build up massively on rutaceous host trees or shrubs in forests [59] but is capable of surviving in low-density populations in natural habitats on sparsely spaced host plants with limited immature flush growth suitable for oviposition and development of nymphs [59]. Liu and Tsai [60] studied development, survival, longevity, reproduction, and life table parameters of ACP in growth chambers at temperatures ranging from 10 °C to 33 °C and relative humidities between 75 and 80%. Populations reared at 10 °C and 33 °C failed to develop [60]. However, populations can flourish in Köppen–Geiger BWh desert and BSh hot semi-arid zones of South Asia, where average daily maximum temperatures over several weeks can reach 45–50 °C [46]. The effects of different temperatures on the life history parameters, including development times and longevity for different geographic populations, have been assessed several times [60][61][62][63][64][65]. A meta-analysis of published ACP temperature-dependent development literature by Milosavljevic et al. [65], synthesising datasets of five globally distributed populations (Brazil, California, China, Florida, and Japan) reared under different constant temperatures on six different host plants (i.e., Rangpur lime, Natal and Pêra oranges, kumquat, mandarin, and orange jasmine), together with the results of the study on Volkamer lemon (*C.* × *otaitensis*), revealed convergence in estimates of developmental parameters. These results have implications for predicting ACP invasions and establishment risks, and subsequent population dynamics across various climactic gradients and geographic regions. Ahmad [66] estimated that >4.5 million adults and nymphs were present in March (spring) 1959 in a one-acre (0.4 ha) irrigated orchard comprising 110 mature *C.* × *aurantiifolia* trees near Multan (30.1981° N, 71.4685° E, 129 m ASL) in the hot-desert Punjab region of Pakistan. Husain and Nath [26] recorded up to 807 eggs per female, but in other studies, fecundity ranged from 180 to near 1900 eggs per female, depending on the ambient conditions of the host plants [60][67][68][69]. Heavy rainfall, particularly monsoon rains, washes eggs and nymphs of plants [46]. Aubert [9][10][11] mentioned that ACP mortality increases with higher rainfall and relative humidity, and that monthly rainfall above 150 mm is associated with low populations due to eggs and young nymphs being washed off plant surfaces.

ACP has been recorded at elevations up to 1250 m above sea level near Kuala Terla (4.5467° N, 101.4162° E) in the Cameron Highlands in Peninsular Malaysia [23][24]. Massive populations were observed on HLB-infected trees in a neglected orchard, but no psyllids were observed on infected trees at 1450 m ASL [23][24]. Om et al. [70] rarely recorded it at elevations above 1200 m in Bhutan and suggested that UV-B, which increases with increasing elevations above sea level, may affect survival of the psyllid. They found no relationship between abundance of the psyllid at elevations ranging from 800 to 1500 m and tree growth, ambient air temperatures, relative humidity, rainfall, or natural enemies [70][71].

4. Natural Enemies of ACP in Asia

Natural enemies of ACP in Asia include the primary parasitoids *T. radiata* and *D. aligarhensis*, predators such as ladybirds (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), ants (Hymenoptera: Formicidae), thrips (Thysanoptera: Phlaeothripidae), syrphid flies (Diptera: Syrphidae), bugs (Hemiptera: Geocoridae), mantids (Mantodea: Mantidae), mites (Acari: Phytoseiidae), and spiders (Acari: Clubionidae, Gnaphosidae and Salticidae), and six species of entomopathogenic fungi [46]. The importance of *O. smaragadina* has been overlooked [72]. It occurs naturally in Borneo and elsewhere in tropical and subtropical citrus regions in Asia, New Guinea, and northern Australia, and has been recognised as an important predator of citrus pests in Asia, particularly China and Vietnam, for some 1700 years [71]. It preys on eggs of ACP, and incidence of psyllid and HLB has been recorded as lower in unsprayed orchards than in orchards sprayed with synthetic pesticides [72][73].

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