Keynote Addresses
K1. KEYNOTE ADDRESS 1: Historical perspectives of HLB in Asia

Aubert B.

Citrus Huanglongbing experiences of integrated vector management (IVM) in Reunion and Guadeloupe, two ultra peripheral regions of the European Union

By the early 1960s Citrus Huanglongbing (HLB) [syn Citrus Greening] increased to the status of a major economic disease both in sub-Saharan Africa and Asia. With the advent of transmission electron microscopy (TEM), Laflèche and Bové (1970) claimed the first identification of the causal agent in the sieve tubes of presumably infected orange leaves originating from South Africa, India and Reunion Island. The HLB organism was the first phloem sieve tube restricted bacterium seen in plants (Bové 2006) with the capacity to proliferate not only in all types of citrus, but also in the hemolymph and salivary glands of two psyllid vectors endemic to either Africa or Asia. Intertropical islands used often as natural quarantine facilities over the previous centuries, were and still are critical steps for the spread of vector borne citrus diseases such as HLB. Biocontrol approaches combined with targeted preventive actions were found relevant for sustainable sanitation in two territories.

HLB and the socioeconomic situation of Reunion Island

Distinctive conditions for HLB epidemics The densely populated territory of La Reunion (currently ~800,000 inhabitants, with a large proportion of migrants from the rim of the Indian Ocean and China), and adjacent Mauritius alike, were facing uncontrolled conditions for HLB epidemics (Moreira 1967 Bové & Cassin 1968, Catling 1973). The reasons were: 1) Presence of the African vector Trioza erytreae thriving without any of its natural parasitoids, and of the Asian vector Diaphorina citri poorly controlled by a single endoparasitic wasp Diaphorencyrtus aligarhensis; 2) High diversity of climates over a small volcanic island of only 2500 km² with steep topography combined with tropical trade winds and occasional hurricanes, thus offering multiple opportunities for vectors to build up and HLB disease to appear; 3) Substantial colonies of ornamental and wild rutaceous plants harbouring the two vectors; 4) Fragmented land ownership of commercial citrus orchards interspersed with countless small citrus plantings and backyard trees; 5) Lack of expedient diagnostic tools for discriminating HLB infection from physiological disorders; and 6) Limited research funding from the Agricultural Research institution IRFA-IRAT-CIRAD in charge.
A patrimonial citrus production likely to exacerbate HLB problem

In Reunion, rainfall, relative humidity and temperature greatly influenced citrus psylla population upsurges, with *T. erytreae* more adapted to high-lying windward cool and wet areas, and *D. citri* favoring dry hot leeward low-lying areas. Depending on the season, overlap of psyllid territories was possible. Furthermore, the intermixing of both types of HLB organisms (i.e. African HLB showing symptoms only in cool climates as opposed to Asian HLB that is less temperature dependant) was enhanced by the capacity of each citrus psyllid to transmit either pathogen. Not surprisingly, individual trees were found to host both strains of agents Ca. *Liberibacter asiaticus* and Ca. *Liberibacter africanus* simultaneously (Gar nier et al 1996).

Taking advantage of the ecological situations, local growers had eagerly cultivated a multitude of citrus types, from deeply coloured mandarins in the mountains, to limes and grapefruits in the coastal areas. This diverse range of commercial plantings was closely intertwined and formed a continuum with small citrus gardens, backyard trees, and urban hedges of *Murraya exotica* orange jasmine. The citrus map, Fig. 4, of present commercial orchards in Reunion, [400ha producing 10.000t of fruit, (Insa et al 2002)] traces back the old struggling situation of the early 1970s when a mere output of 800t was obtained. At that time tree declines due to HLB epidemics were typically compensated by accelerated cycles of orchard replanting with unsafe nursery material. Under such conditions, any attempt of compulsory eradication for citrus and citrus-relatives in Reunion appeared socially and technically unbearable.

Strategies of sustainable sanitation based on integrated vector management (IVC)

In line with the recommendations of the International Organization of Citrus Virologists (IOCV) especially during a post-conference tour taking place after the 6th congress held in Mbabane, Swaziland 1972, and initiated by J. M. Bové, the following strategy was decided:

**Incentives for replanting certified disease-free material**: Registered budwood, free of graft transmissible diseases, was received from SRA San Giuliano Corsica in 1969. New cultivars, especially easy peelers exhibiting attractive qualities, were propagated within strict registered nursery requirements (up to 35,000 trees/year). To enhance the removal of affected commercial orchards, local authorities (Chambre d’Agriculture de la Réunion) refunded the cost of replanting and trained extension service personnel to survey/assist a new generation of growers aware of the benefits of preventive sprays. Although uncertain in terms of prognosis, this strategy was nevertheless considered more appropriate than a costly and hazardous eradication scheme. Such a propagation system of certified planting material, first initiated in 1969, is currently operating with modern insect-proof and full covered greenhouses delivering virus-free and citrus-canker-free planting material. This compulsory CAC-EU label: *conformité agricole communautaire*, is now required since Reunion is an ultra-peripheral region of the European Union.

Fig. 5: Tullus Ltd. container-grown citrus nursery in Reunion (courtesy M. Roux Cuvelier CIRAD Réunion Sept. 2008).

Biological control of the vectors: Concurrently, a specific biological control program was launched in 1974. Its aim was the introduction, rearing, and mass release of primary parasites of psyllid nymphs (Order Hymenoptera superfamily Chalcidoidea,). Primary ectoparasitic wasps *Tamarixia* spp were found much more efficient than primary endoparasitic *Psyllaephagus* sp. or *Diaphorencyrtus* sp. Emphasis was therefore put on the African *Tamarixia dryi* which originated from Nelspruit South Africa and on the Asian *Tamarixia radiata* which originated from Badal, West Punjab, India. For the former wasp, 33,000 adults were released on the island thus corresponding to 50 adults per km² of citrus area, and this was followed by a release of 3,500 adults in the restricted citrus area of the dry lowlands where *D.citri* was predominant.

A careful elimination of secondary or tertiary parasitoids enabled these wasps to establish properly and resulted in a drastic reduction of vector populations within 3 years (Etienne and Aubert 1980).

The two primary ectoparasitic wasps have similar biologies and show remarkable host-searching ability. The females lay eggs on psyllid nymphs of the 3rd, 4th and 5th instars. Their life cycle is only 12 to 14 days as opposed to 21 days for endoparasitic wasps. Being ectoparasitic, the larva feed on and suck out the hemolymph of the psyllid nymphs. Adults pupate in the mummies of the nymphs and emerge by chewing a hole through the thorax of the psyllid host. The size and shape of the hole, and the meconium left by the parasitoid in the nymph mummy give a signature of the absence of secondary or tertiary parasitoids. Taxonomic studies related to this biocontrol were carried out with the assistance of the Museums of Natural History of Pretoria and London (Annecke et al 1971, Prinsloo 1981 and Hollis 1984) and with information on hyperparasites available from Husein and Nath (1924).

The primary endoparasitic *Psyllaephagus pulvinatus*, imported into Reunion from South Africa, was largely outcompeted by *T. dryi* and disappeared. *Trioza erytreae*, easily spotted by its remarkable formation of galls left by feeding nymphs on citrus leaves, survived for six years on
semi-wild lemon plants at 900m elevation in an extremely wet area, but was eventually completely eliminated. The only explanation for this unexpected result is the build-up of *T. dryi* on an alternative polyphagous psyllid *Trioza litseae* (Hollis 1984), occasionally feeding on avocados, citrus, vanilla and papaws, but predominantly on a common aromatic shrub *Litsea glutinosa*. This plant originated from Asia and Australia, was imported into Reunion many years ago, and was largely disseminated by local birds. *T. dryi* ex *T. litseae* was found to be conspecific with *T. dryi* ex *T. erytreae* (Prinsloo unpublished). In Eastern and Southern Africa, *T. dryi* is apparently unable to parasitize any psyllid other than *T. erytreae*. Neither *T. litseae* nor *Litsea chinensis* occur in Africa, both having an Asian origin. Relative to the Asian citrus psyllid, *D. citri*, it survived on pruned hedges of *Murraya exotica* but was rarely seen on citrus, even on neglected trees. *T. radiata* was unable to parasitize any psyllid other than *D. citri* (Aubert and Quilici 1984), and further laboratory studies were conducted for disclosing its basic life-history traits (Fauvergue and Quilici 1991, Quilici 1992).

**Symptomatology.** Experimental transmissions of HLB by grafting were performed under screen house conditions to track mineral-like deficiencies induced by the disease, as well as phloem disorders. These investigations consisted of both healthy and HLB-affected trees and were useful to accurately assess visual symptoms, especially the sectoring of blotchy mottle plus Mn and Zn deficiencies that preceded twig dieback. A canopy rating method was subsequently developed for field evaluation of HLB. For this rating, individual trees were divided into eight quadrants, and each quadrant was assigned a value of 0 to 5 for increasing symptoms of HLB severity. Thus an individual tree could have a combined HLB severity rating of 0 to 40. Any tree beyond quotation rating 25 was considered as commercially nonviable. Extension service personnel were trained to evaluate the severity of HLB symptoms using this canopy rating system.

**Disease aetiology and antibiotic injections.** Considering the tedious and costly diagnostic procedure of sampling trees and assaying via TEM (leaf midribs or fruit columella dispatched to INRA Bordeaux in 2% buffered glutaraldehyde), a field experiment was initiated to confirm the bacterial infection of HLB epidemics. Antibiotic injections were tested in a 6-year-old orange grove (one hectare) leased from a private sugar estate (Sucreries Bénard du Gol). Although planted in 1971 with SRA disease-free material, and established in the middle of sugar cane blocks with preventive insecticide sprays, these orange trees were inoculated via psyllid at early stage of planting. The trial comprised eleven triplets of trees with the same HLB canopy rating. The triplets comprising a total of 33 trees were injected once a year during the main flush season for three years (October 1977, 1978, and 1979) with penicillin, tetracycline, or plain water, respectively. Canopy rating was assessed once a year after the main flush, and fruit harvested for yields and weights just before full maturity to avoid yield losses due to theft. The individual tree dosage of penicillin was 100mg per kg of fresh weight, *i.e.*, for 6-year-old trees an injection of 18g diluted into 6 liters of water. For tetracycline 35mg per kg of fresh weight was chosen, *i.e.*, 6g diluted into 2 liters of water. The pressure for injections was 5kg/cm². No phytotoxic effect...
was noticed with penicillin, while tetracycline induced brief willow-leaf symptoms prior to stimulating vigorous healthy-looking regrowth. Concerning penicillin, antibiograms performed on crushed leaf-midribs after the onset of tree injection showed that bacteriostatic effects in orange leaves lasted for 120 hours.

The results of this field experiment were the following: 1) significant canopy recovery was followed by yield increase with penicillin injections, and 2) similar but longer beneficial effect of tetracycline injections. The recovery with penicillin treatments implied that the origin of the disease was the infection by a gram negative bacterium, since this antibiotic is hindering the formation of peptidoglycane layers of the bacterial cell wall, as demonstrated conclusively by Garnier et al., (1984) on graft-inoculated young orange seedlings treated by root drenching with penicillin.

But neither penicillin nor tetracycline resulted in long-term suppression of HLB, probably due to the bacteriostatic rather than bactericidal effect of antibiotics and possible developments of resistance. This experiment also demonstrated how rapidly treated trees relapsed following the second and third injections, a clear indication of the virulent character of the HLB organism.
Epidemiological survey. Temporal increase of HLB disease was evaluated by visual canopy assessment on two groups of commercial orchards amounting to 1000 trees each, with the assistance of trained extension service personnel. Group A of orchards was planted in 1970/71 before the biological control program, and evaluated in 1975, 1977, 1979 and 1980, respectively. Group B planted in 1977/78 after the biocontrol program of psyllid vectors was similarly evaluated respectively in 1981, 1984. Comparative rates of spread of the disease were evaluated following Van der Plank’s analysis. In group A, 50% of the trees were commercially lost seven years after planting, while in group B disease rate was so low that 50% loss would theoretically occur around 2015. Gottwald et al, (2007) recently reviewed the epidemiology of HLB by comparing Reunion, Chinese, Brazilian and Florida situations.

Conclusion on the Integrated Vector Management story of Reunion

In spite of an original hopelessly massive psylla build-up and spread of the pathogen, substantial results for controlling the HLB disease were obtained in a rather short time for a problem affecting perennial plants. The training of extension service consultants for monitoring the disease, and training/educating the farmers were important aspects of the program. As stressed by Ohmart (2008) integrated management cannot succeed without the awareness of the growers. The new generation of orchards sprayed with horticultural oil for controlling other insects and mites, resulted in an extremely low rate of disease progression. A common practice adopted by the farmers in commercial orchards and by many residential owners of small gardens and backyard trees, was then to voluntarily remove affected trees and replant. Today HLB is a forgotten nightmare in Reunion, to the point that any research on this dangerous disease is being discontinued. However, accurately detecting the last HLB foci for their eradication would be wise.

Neither hurricanes nor the strange episode of rural hyper epidemics of human ‘chikungunya’ virus disease transmitted by A. albopictus in 2006, had any marked effect on the new citrus psyllid ecosystem,
notwithstanding massive emergency applications of fenithrothion and deltametrine to eliminate the adult mosquito vectors of this human disease. The major challenge for the citrus growers now is the replacement of old healthy trees established over the past 30 years, with novel cultivar selections that have better prices on the local fresh fruit market.

With as much as 130 Corine biotopes and a rich flora including 600 endemic species and some 2400 imported/introduced ones, Reunion is considered a hot spot of world biodiversity. This may explain the success of the psyllid vector biocontrol. Similar results were obtained in the neighbouring Mauritius island, where the Reunion approach was duplicated concomitantly.

Integrated Vector Management in Guadeloupe

The first detection of \textit{D.citri} in the Caribbean island-chain, occurred in Guadeloupe on backyard orange trees in January 1998 (Étienne et al, 1998). After one year of investigation, no parasitism was found on these intrusive \textit{D.citri} colonies, and the introduction of \textit{Tamarixia radiata} from Réunion was decided in January 1999 (Étienne et al., 2001). The rearing and release of a few hundred wasps succeeded in establishing the ectoparasitoid and noticeably reduced \textit{D.citri} populations not only on citrus backyard trees and \textit{Murraya exotica} hedges, but also over the 360 ha of local commercial orchards of limes, oranges and mandarins. This program of biocontrol was developed together with three other biocontrol programs, targeting newly arrived citrus pests appearing suddenly in the late 1990s and for which specific wasps were also imported, i.e. \textit{Anagyrus kamali} against the hibiscus pink mealybug, \textit{Ageniaspis citricola} against the citrus the leaf miner, and \textit{Lysiphlebus} sp. against the brown citrus aphid. In April 1998, Guadeloupe sent a warning to the entomology department of UF-IFAS Homestead, and three months later \textit{D.citri} was detected in South Florida (Knapp et al., 1998).

Today, ten years after the preventive biocontrol launched against \textit{D.citri} in Guadeloupe, the HLB organism has not yet been detected there, and the neighbouring Martinique and La Dominique islands are still free of \textit{D. citri}. In the Greater Antilles, the Asian vector of HLB was found in Cuba in 1999, then in Haiti in 2000, in the Dominican Republic in 2001, in Puerto Rico in 2002 and in Jamaica in 2003 (Halbert and Nunez, 2004). Besides Guadeloupe, \textit{T.radiata} is now present in Florida, Cuba and Puerto Rico. It has to be seen what will result in terms of vector control and HLB spread for the latter territories, depending on local ecosystems, specific ectoparasitoid introductions, and strategies of integrated management.

Other recent island situations

The accidental introduction of \textit{Trioza erytreae} in Madeira in 1994 (Fernandes & Franquinho 2001) and its extension some years later into Tenerife, Gomera & Palma Canary Islands (Perez Padron & Hernandez 2002), is a new threat for the citrus production of the western part of the Mediterranean Basin. Similarly the presence of \textit{D. citri} on the islands of Hawaii and Maui (Conant et al 2007) and the interception in 2008 of the Asian HLB vector on ornamental rutaceous plants (curry plant) dispatched from there to California is an additional example of the need for relevant surveys and controls in island situations.

The sudden spread of HLB in citrus-producing areas previously regarded as HLB-free, highlights the potential threat of one of the most serious diseases of citrus. Considering the extreme fertility
of both psyllid vectors with each female laying as many as 1000 to 2000 eggs in a matter of 3 weeks, chemical protection alone may end in a vicious cycle with rising levels of resistance and damage to the environment. Boosting carefully screened natural enemies and helping farmers to learn the dynamics of their ecosystems may offer interesting alternatives.

**Literature cited**


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