British Society for Plant Pathology

Huanglongbing

A century-long citrus pandemic

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PLANT PANDEMICS 1

Short Study Awards

This is one of a series of case studies, sponsored by the British Society for Plant Pathology, on damaging plant diseases which have had – and continue to have – major economic, social and environmental impacts around the world. The case studies provide an historical overview of how scientists have responded to plant pandemics and the evolution and effectiveness of management strategies. The purpose of the reports is to raise awareness of plant pandemics and to stimulate wider interest in their consequences for all, including current and future researchers.

Copies of the report are available from the BSPP | www.bspp.org.uk.

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Cover photo

A farmer brings a sample from a sick and ailing orange tree to a plant clinic in Sri Lanka – one of many countries under threat from Huanglongbing disease. Photo Eric Boa

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Introduction

Citrus fruit are a ubiquitous part of our diet: breakfast orange or grapefruit juice, satsumas in a lunch box, and lemons and limes to flavour our savoury dishes or to garnish drinks. In the UK, citrus fruit are second only to bananas in popularity , famously contributing vitamin C, fibre and micronutrients to our diets. In Northern Europe, all of our citrus fruit is imported, because the trees will not tolerate winter frosts, and flourish best in the sunshine and warmth between 40° N & 40° S (Fig. 1).

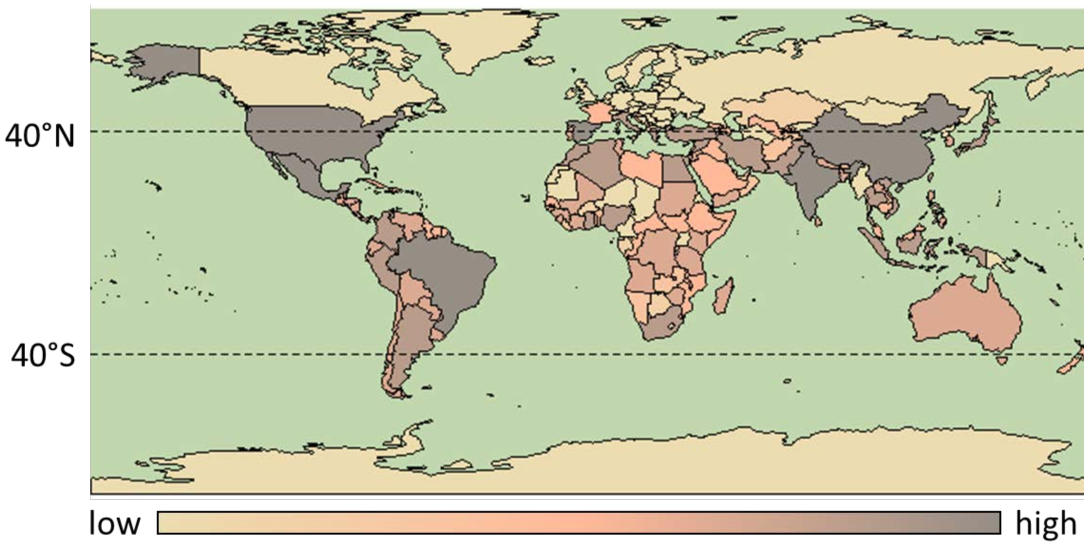

Figure 1: Global distribution of citrus production 2018. (FAOSTAT)

We may not, therefore, be aware of the skill and challenges involved in growing these sensitive and valuable fruit. Citrus growers were already struggling with diseases such as bacterial canker and tristeza before the onset of the most serious pandemic to hit the citrus industry: huanglongbing (HLB) or citrus greening.

The spread of HLB affects not only fruit availability to consumers, but more seriously impacts communities and industries that rely on high yields of citrus fruit to survive. Worldwide, 152 million tonnes of citrus fruit are grown every year (Figure 2), worth USD 2 billion.

In citrus-growing regions, like southern China, the Mediterranean and Florida, the fruit are not only grown and consumed, but form a fundamental part of a regional identity, and keep rural communities alive through income and employment. Since HLB infection spread to North America, their citrus production has dropped by 50% (Figure 3).

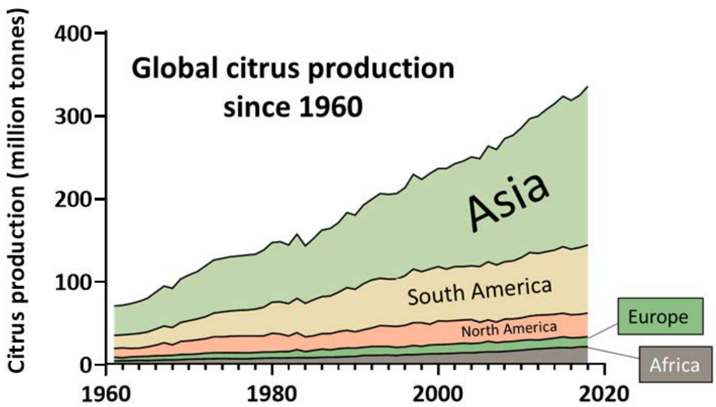

Figure 2: Growth of global citrus production from 1960-2019 (FAOSTAT, 2018)

In the main orange growing regions of China and Brazil, HLB has reduced tree numbers by 10-30%. Such a devastating citrus disease on a global scale has caught the attention of governments and scientists: the US government alone has spent 400 million USD to research HLB. And yet, there is no cure, and the disease continues to spread. So why is the disease so intractable?

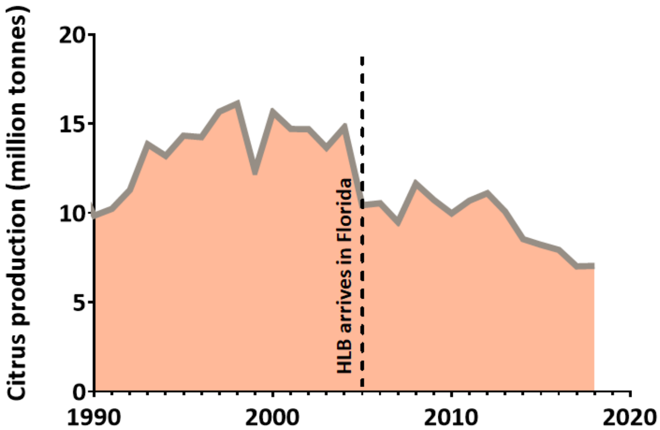

Figure 3: Decline in citrus production in North America since the detection of HLB in Florida (FAOSTAT, 2018)

Huanglongbing is an elusive disease: it has eluded identification, classification, treatment and elimination for the last century. There are two major types of the disease: African and Asian. We will explore the spread of HLB from early 20th century East Asia, across Africa and into the Americas, and tell the stories of the communities and scientists who have tried to understand HLB and limit its impact. Although the disease has many local names, particularly 'greening', we will refer to it throughout by its official name: huanglongbing or HLB.

What does Huanglongbing look like?

In the early 20th century, well before huanglongbing was identified as a single disease, there were reports of its symptoms accumulating in China, India, the Philippines, and South Africa. The symptoms of HLB found 100 years ago are the same as those found today. Farmers and agricultural scientists were encountering an unusual blotchy mottle: irregular patches of chlorosis along the leaf veins, followed by a reduction in quality and quantity of fruit (Figs 4 and 5). Further development of the disease resulted in stunted branches and thick, corky veins. Increasing leaf drop and defoliation was followed by dieback of the infected branches. The roots of infected trees were also stunted.

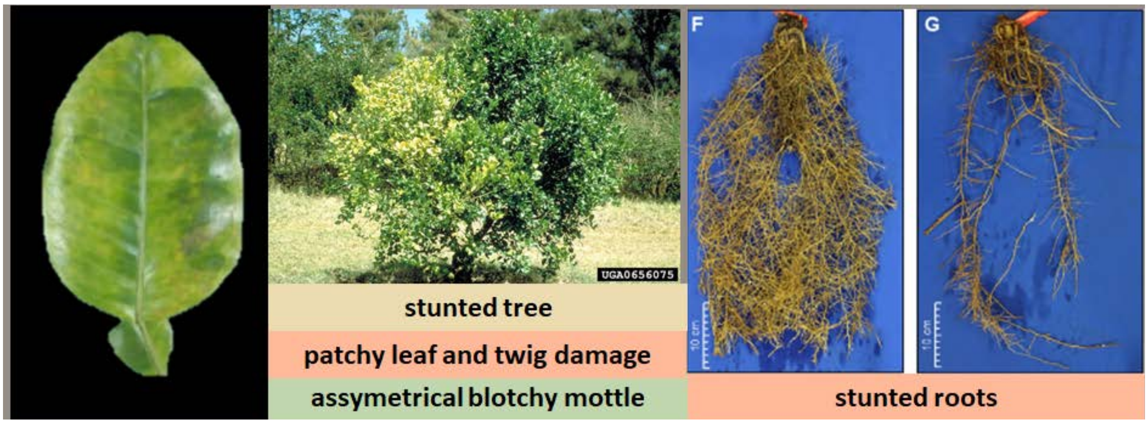

Figure 4: Symptoms of HLB on infected citrus plants

The individual symptoms of HLB resemble those of many other citrus afflictions: nutrient deficiency, waterlogging, nematode damage, and other citrus diseases, such as the virus tristeza. It is unsurprising that the causal agents of HLB were not immediately identified. The first scientists to search for the cause of the damage to so many citrus groves were based in the home of citrus: China and India.

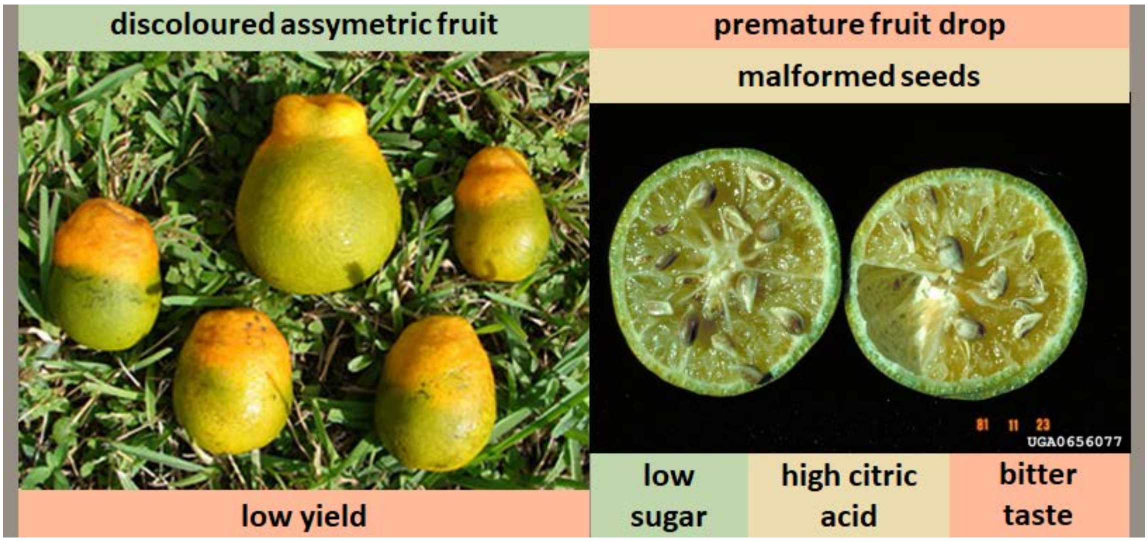

Figure 5: Symptoms of HLB on fruit. These include asymmetric development and discolouration. Fruit of infected plants also usually have malformed seeds

Lin Kong-Hsiang and the rise of Huanglongbing

The main citrus fruit that we eat today (oranges, lemons, grapefruit) are all derived from just a few, less famous, ancestral species. This close family relationship means that HLB is able to infect all citrus varieties , as well as some citrus' more distant ornamental relatives such as orange jasmine and boxwood.

Mandarins and sweet oranges are among the most affected by HLB; lemons and limes are slightly less susceptible (Fig. 6).

These ancestral citrus species developed at the base of the Himalayas, in present day China and India. Accordingly, this is where we saw the first reports of HLB in the late 19th and early 20th century. In the 1890s, in both India and China, farmers were reporting yellowing and mottling of the leaves of their citrus trees, and a decline in fruit production.

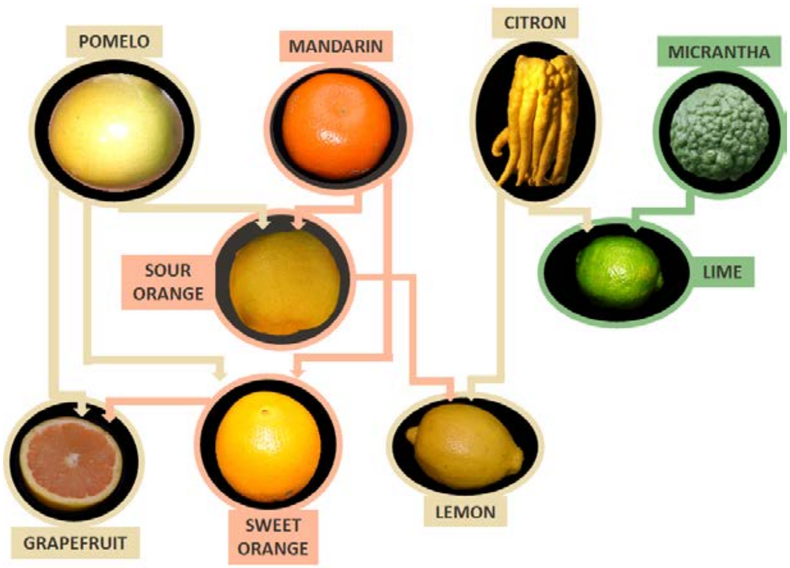

Figure 6: Citrus family tree. Adapted from Wu et al., 2018. Nature, 554 (7692).

The Pearl river (Zhujiang) draws together 2400 km of tributaries across southern China, and empties into the South China Sea between Hong Kong and Macau. The watershed of the Pearl River is the heartland of Chinese citrus production both today and in the early 20th century (Fig. 7).

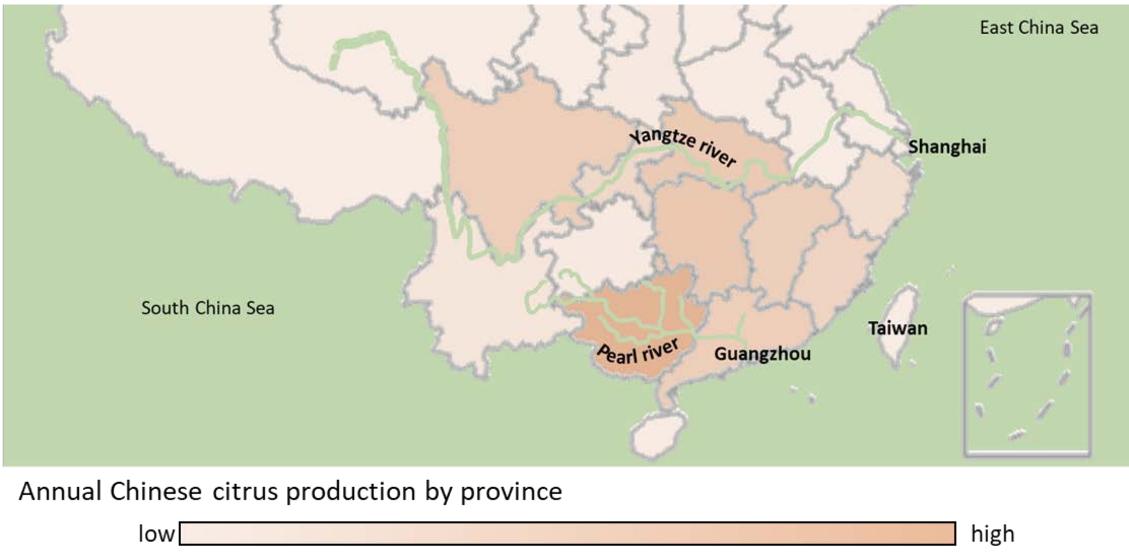

Figure 7: Annual citrus production in China

The father of HLB research

Plant pathologist Professor Lin Kong-Hsiang (林孔湘) (Fig. 8) was born in the Pearl River watershed in 1910 on the south coast of China overlooking the island of Taiwan. At that time, the Qing emperor still ruled China, and farmers were already reporting symptoms of HLB throughout the Pearl River watershed. Professor Lin would provide the foundation of the global scientific community's understanding of HLB, despite the many hurdles of conducting science in mid-20th century China.

Figure 8: Professor Lin Kong-Hsiang

Today, it is difficult to imagine the upheavals in agriculture, science and society that Professor Lin and his colleagues witnessed throughout their careers: civil war; invasion; agricultural restructuring; famine; politicisation of science; closing of universities during the Cultural Revolution; and a general limitation on engaging with international scientific colleagues. Much of the early, important work on HLB by Lin and many other Chinese academics was never seen by the outside world and remains untranslated.

Wartime HLB research in China

Lin returned to China in 1941 following his PhD at Cornell in the US, took up a post at the prestigious Lingnan University in Guangzhou, and began a 2600 km survey of citrus plantations in southern China. HLB had become widespread. However, Japanese forces had invaded Guangzhou in 1939, and the occupation coupled with local famine caused the death or displacement of up to 3 million people in Guangdong province. Citrus pathology was not a top priority at this time; indeed, citrus groves were dug up to increase rice planting to feed the starving province during the war. When the groves were replanted in the post-war period, the more vulnerable new trees were diseased within seven years.

By 1947, Prof. Lin had restarted his research into the cause of HLB, despite the ongoing civil war. He produced a seminal paper in 1956, which addressed all the theories behind the causes of HLB3. His experiments for this one paper included planting over 3000 citrus trees and interviewing farmers for fifteen years in several thousand km2 of citrus groves.

Under normal circumstances, this would be a significant piece of work; 1950s China saw an upheaval in agriculture which surpasses anything experienced at any time elsewhere in the world. At the beginning of the fifties, the Agrarian Reform Law came into force in China, which redistributed all privately owned land to farmers. By the mid-fifties, farms were collectivised, quotas were created, and crops were dictated. By the end of the decade, a combination of these agricultural policies, droughts and flooding led to a 30% decline in crop yields, and years of famine, which killed tens of millions of people. Citrus fruit were not a priority.

Professor Lin versus the Soviet scientists

In the early 1950s, when Lin was carrying out his studies, the Chinese Communist Party (CCP) was heavily reliant on Soviet technology and funding. The deeply flawed theories of Soviet agronomists, such as Michurin and Lysenko, were officially sanctioned by the CCP; dissent and even Mendelian genetics were outlawed. In 1952, Soviet scientists visited Guangzhou to investigate the cause of HLB. They told Lin that his experiments were flawed, which was why he had been unable to find the cause of HLB. The Soviets insisted that HLB was caused by waterlogging, and the situation could be rectified by moving the citrus groves uphill to better drained areas, so this is what the farmers did.

The situation did not improve. By 1954, Lin had conducted his waterlogging experiments (Figure 9), which showed the difference between HLB and waterlogging symptoms. He explained his theory of the disease in papers, at conferences, but the agricultural academies and policies would not change, and he faced much criticism. It took until 1965 before the Ministry of Agriculture finally accepted his evidence, by which time many thousands of trees had been destroyed, new groves had been planted, and succumbed to HLB.

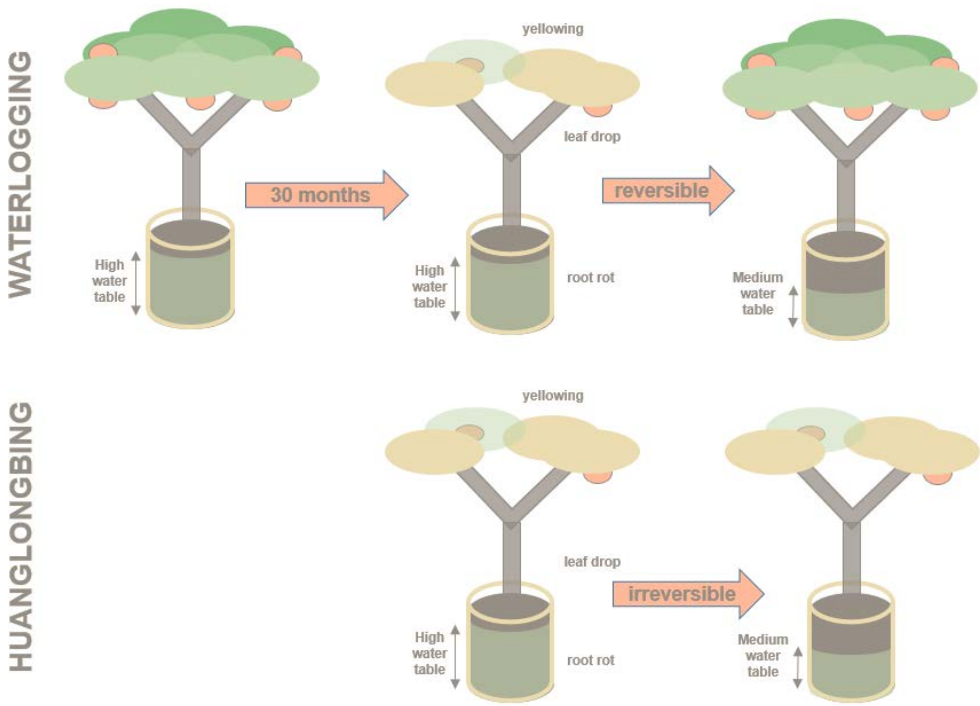

Figure 9: Lin's waterlogging experiment showed that HLB was not caused by high water tables

The many experiments of Professor Lin

Lin did extensive experimentation to prove that HLB was not caused by a fungus, nematodes, waterlogging, nor nutrient deficiency. He systematically grew mandarin trees and exposed them to the different biotic and abiotic stresses that other scientists had proposed were behind the HLB symptoms, and detailed the difference in impact on leaf and fruit compared to HLB. This did not make him popular with his colleagues.

HLB suspected of being a viral disease

One scientist with whom Prof. Lin was in agreement was Chen Qibao [陈其傑] who had begun grafting experiments twenty years earlier during the Sino-Japanese war, and concluded that HLB was graft-transmissible (Fig. 10). At this time, the only known graft-transmissible organisms were viruses. Scientists had only seen a virus for the first time in 1931, and the International Conference of Citrus Virologists would only be established in 1957. This was a nascent field. Prof. Lin's 1956 paper expanded on Chen's work by reproducing the graft experiments, but also observing the rate of infection of surrounding uninfected trees.

The grafting of HLB-infected budstock caused systemic disease symptoms within 1-2 years, whereas some uninoculated trees were just beginning to show HLB symptoms by this time. Lin concluded from this that HLB was possibly spread by an insect vector and graft-transmissible, therefore likely caused by a virus. Unbeknownst to Lin, ten thousand kilometres away in Pretoria, two South African scientists were conducting the same grafting experiments, and drawing very similar conclusions.

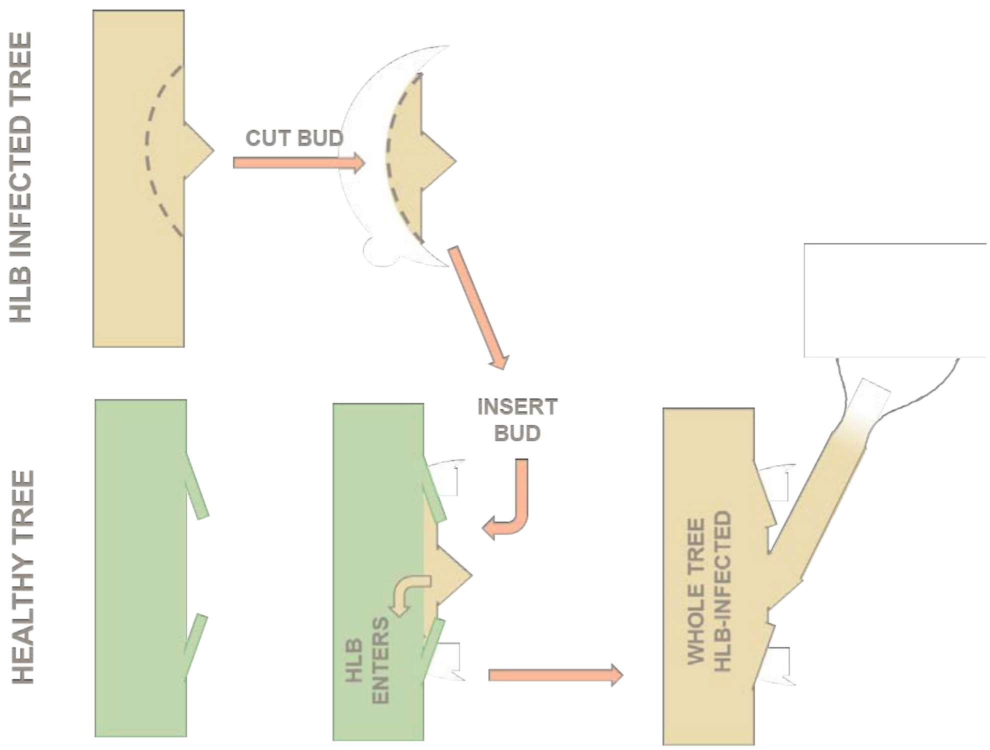

Figure 10: Lin and Chen's experiments showed that HLB was graft-transmissible.

The arrival of Huanglongbing in South Africa

In the University of Pretoria, the horticulturalist P.C.J. Oberholzer and his plant pathologist colleague A.P.D. McClean published results of their own grafting experiment in 1965. They were investigating the cause of the HLB symptoms that had caused severe citrus crop losses of 30-100% in in the eastern citrus-growing regions in 1932-36 and 1939-46. These symptoms had first been noted in South Africa in 1929, and were given the name "greening". Greening was causing problems with the significant export industry that South Africa had developed.

The South African citrus export industry thrived owing to its counter-seasonal advantage in northern markets: South African fruit is mostly harvested May-August, whereas European and Chinese citrus fruit is ripe in the winter months. The coastal provinces of South Africa are all climatically suitable for citrus trees, with high humidity and low risk of frost (Fig. 11).

The industry initially began in the Western Cape region in the 17th century, when the Dutch East India Company brought orange trees from St Helena to help combat scurvy in visiting mariners. Settlers planted the trees throughout the country in the 19th century, but it was not until 1902 that the first export to England heralded a citrus industry boom. By the early 20th century, South African citrus was mainly produced for fresh export to Northern Europe, which demanded high quality fruit.

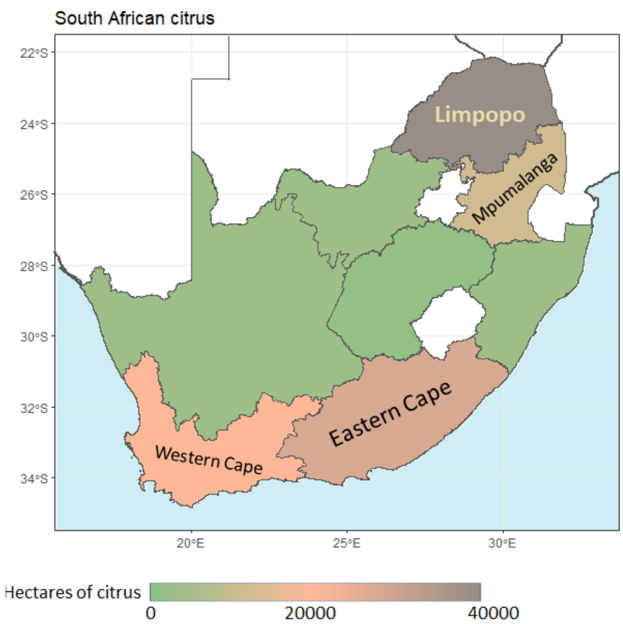

Figure 11: Citrus production in South Africa

By the 1950s, the required quality of the South African fresh fruit was being compromised by the spreading HLB, particularly in sweet oranges. Although the symptoms of 'greening' in South Africa were very similar to Lin's Huanglongbing, the climatic range of the disease was different, and the insects feeding on South African citrus were not found in East Asia. It was not clear to the researchers involved that they were dealing with the same disease until they joined forces. However, Oberholzer and McClean also concluded from their grafting experiment, that the HLB symptoms in South **Africa** were caused by a virus.

The First Conference of HLB experts

So prevalent was the HLB-virus theory that HLB has been on the agenda of the International Organisation of Citrus Virologists (IOCV) since its inception 63 years ago. At that time, pre-internet and cheap air-travel, communication between scientists was more difficult. This was particularly true for those in other parts of the world, who spoke different languages. Conferences served as an important confluence of expertise and knowledge.

The IOCV conferences initially only brought together scientists from 12-15 countries who could afford to travel to the USA. However, the few experts from outside the Americas who did present there, particularly those from East Asia and South Africa, were able to compare and contrast the diseases affecting their local citrus groves.

It was here that experts began to disentangle the difference between zinc deficiency, tristeza virus disease, stubborn disease, the different local names given to HLB, and the different varieties of HLB. These local names for HLB persisted until 1995, when the IOCV agreed to use Prof Lin's original nomenclature: huanglongbing. It was not until microscopy became advanced enough in the 1970s that these scientists were able to identify the causal agents, which were not viruses at all.

Orange plantation, Western Cape, South Africa

Huanglongbing under the microscope

From virus to mycoplasma

By 1970, scientists were using electron microscopy to identify very small single-celled organisms in the leaves of HLB-infected plants. These organisms were initially thought to be mycoplasma-like organisms , which are the smallest known form of bacteria, characterised by a lack of cell wall, and thereby resistant to antibiotics. Scientists from East Asia, India and Africa used the same technique to look at their local samples, and were able to identify that they were all one and the same. Scientists were a big step closer to confirming the causal agent and vectors of HLB.

From mycoplasma to new type of bacteria: Candidatus liberibacter

There were many doubters of the mycoplasma nature of HLB, particularly due to the slight improvement antibiotic injection made on HLB-infected plants. However, it was not until 1984 that microscopist Monique Garnier demonstrated what we know today: that HLB is caused by a bacterium with a cell wall (Gram negative) that is found in the phloem sieve tubes of infected plants(Figure 12). Prof Garnier identified that both the African and Asian forms of the bacterium were similar, and placed both in the Candidatus Liberibacter genus. Candidatus means that the bacteria cannot be maintained in culture in the lab. This made studying the disease, and testing treatments, much more time-consuming.

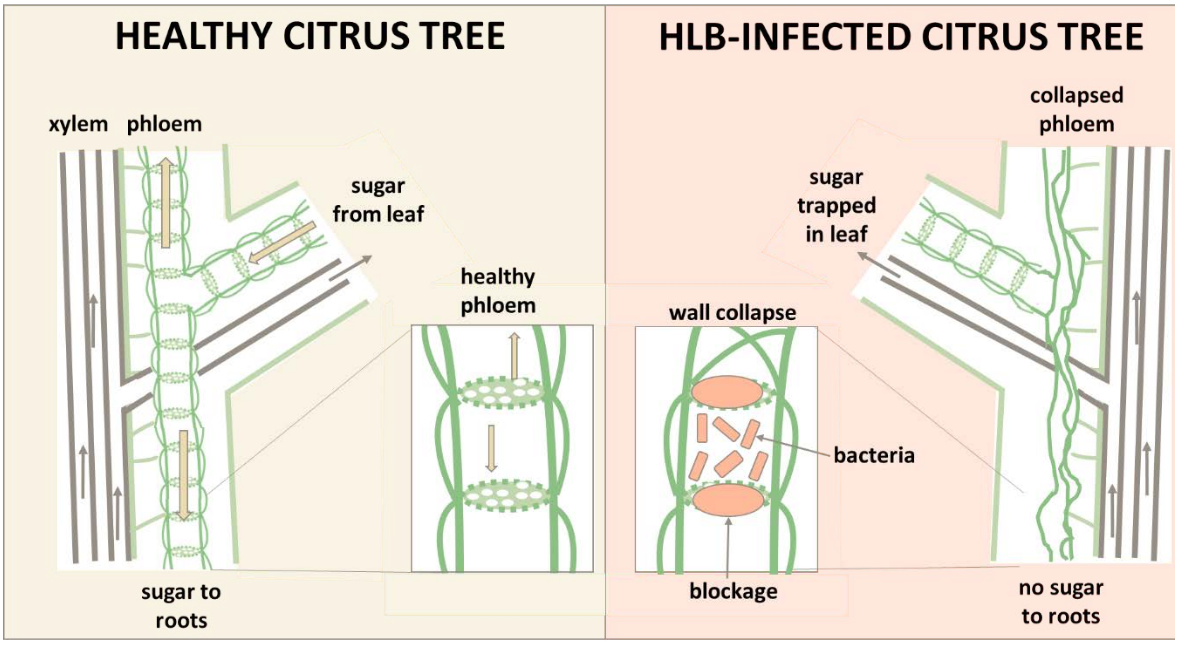

Figure 12: The impact of HLB on the phloem and leaves of infected citrus trees

We now know that there are three main species that can infect citrus plants: Candidatus Liberibacter asiaticus, africanus and americanus, shortened to CLas, CLaf and CLam respectively. Prof. Garnier and her colleagues sampled infected citrus from around the world and began to map the true extent of huanglongbing. Liberibacter species have been detected globally and are present in every continent except for Europe and Australasia (Figure 13).

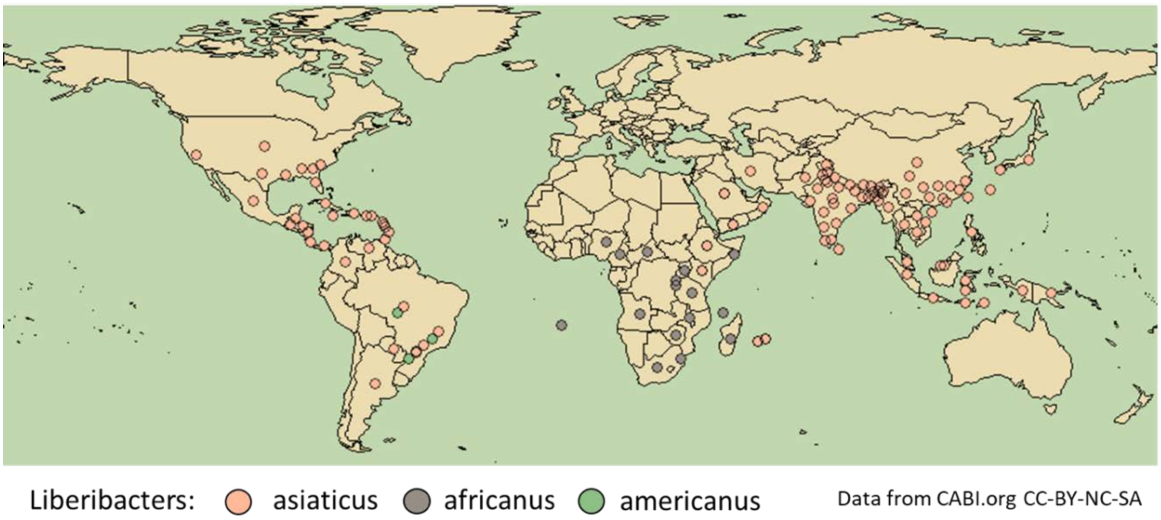

Figure 13: Global distribution of Candidatus Liberibacter species

As part of understanding the global variation in HLB's causal agent, a Luxembourger plant pathologist called Joseph Bové visited South Africa in 1968. Bové found out from researchers there that the HLB disease symptoms were originally detected at higher altitudes, or at low altitudes with a cool climate , and that there was reduced HLB symptoms of oranges grown in warmer fibreglass cages.

Back in Paris, Bové and his colleagues began temperature-controlled experiments on oranges trees grafted with buds from infected trees from India, the Philippines and South Africa. These experiments demonstrated the difference between the two strains: African HLB only flourished at ≤ 24°C, but the Asian HLB was tolerant of heat up to at least 32°C. These temperature ranges matched those of the corresponding insect vectors, citrus psyllids.

The Spread of Huanglongbing

Identification of how the disease is spread

The bacteria that cause HLB cannot move between plants without help from humans or insects. Humans provide the long-range distribution of the disease through grafting and planting of infected material, and insects provide short and long range spread and continuation of the disease.

Short-distance spread of HLB

Psyllids had long been identified as pests of citrus trees. They lay their eggs on the new flush of leaves, and can cause damage to the leaves with their feeding and egg development alone. HLB spread is facilitated by the Asian citrus psyllid, *Diaphorina citri*, and the African citrus psyllid, *Trioza erytreae* (Fig. 14). *D. citri* was first detected in Taiwan in 1907 and *T. erytreae*, which is native to SE Africa, was originally described in Eritrea in 1918 and was later recorded in South Africa in 1929. The presence of these psyllids strongly correlated with areas of high HLB symptoms (Figure 15), and the Liberibacter were identified in their mouths and salivary glands.

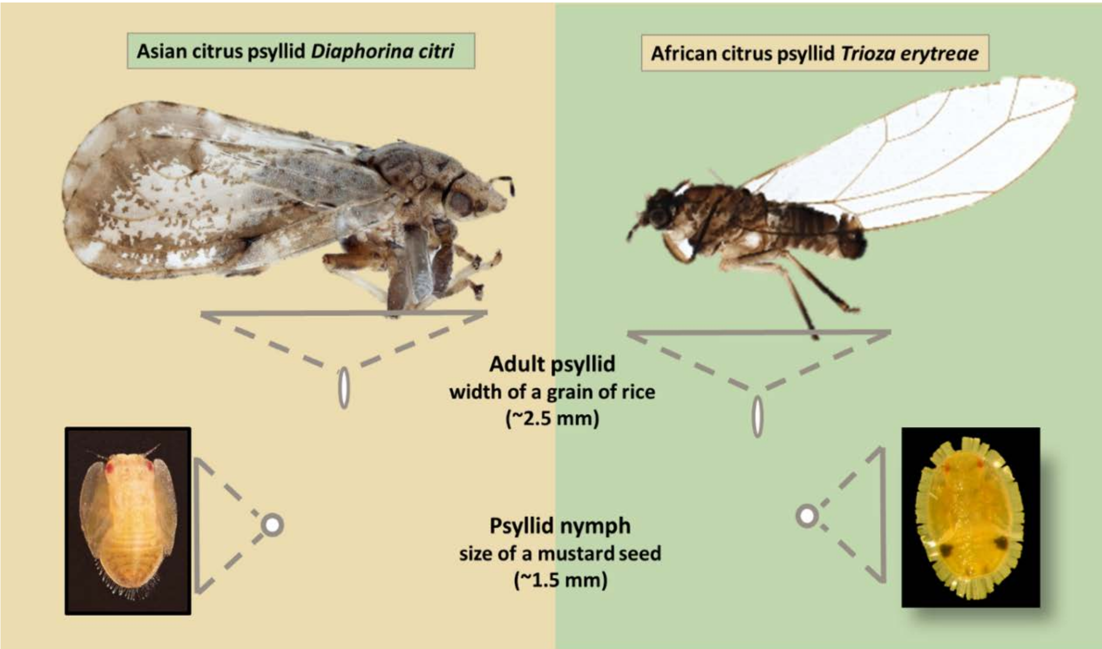

Figure 14: Asian citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*) adult and nymphs

Long distance spread of HLB

Citrus trees are usually propagated by grafting a bud from the tree that produces the desired fruit with the rootstock of a tree with good growth and disease-resistance in local soil. Traditionally, when new groves were being established, budstock would be brought in from a nursery in a successful citrus-growing region, such as China. New varieties of citrus often only have one original source. As a result, genetically identical plants spread globally, and took any diseases they contained with them. As Prof Lin demonstrated in the 1950s, the HLB bacteria are able to travel across grafts, and infect the whole plant. This was the cause of the initial long-distance spread of HLB, until the industry realised the need for HLB-free propagation. HLB may arrive in grafted material well before the insect vector is present, but it would be unable to spread rapidly until the psyllids become established.

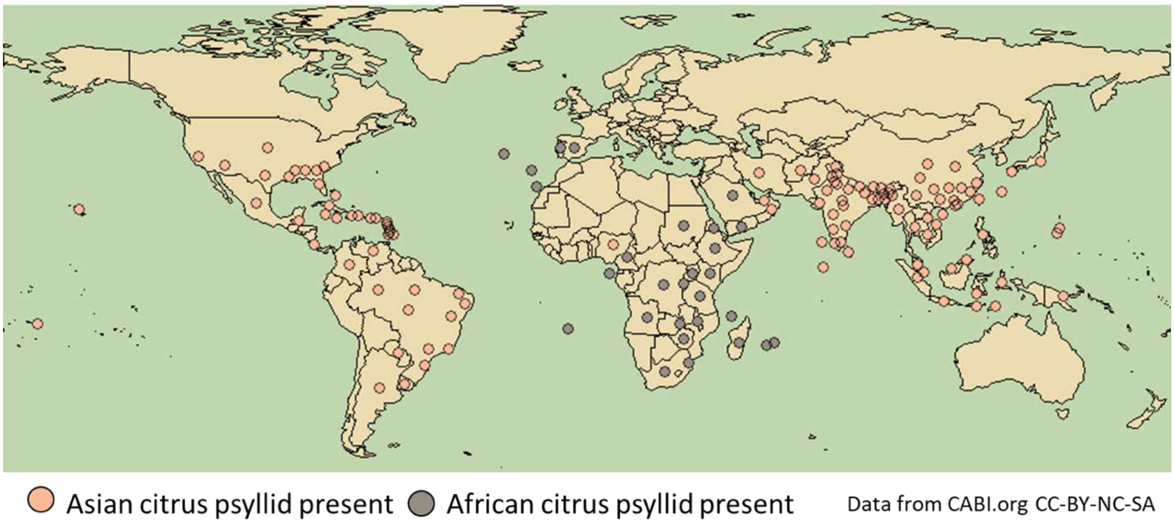

Figure 15: Global distribution of the HLB psyllid vectors, the Asian citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*)

There is also evidence to suggest psyllid-mediated, long-distance spread of HLB. Although the psyllids that spread the disease are unable to fly very far, it is thought that over a succession of short flights *D. citri* can travel up to 2 km within 12 days . Extreme weather can also carry psyllids long distances. In Florida, hurricanes and tropical storms may have carried psyllids in air currents over the Everglades, where there are no citrus orchards, to uninfected farms. Hurricanes can also cause damage to citrus trees resulting in new growth and an increase in psyllid populations, providing the perfect conditions for the disease to spread.

How do psyllids spread the disease?

Adults psyllids feed on the sap of plants in the Rutaceae family, to which all citrus trees belong. They tend to feed on young stems and leaves at all stages of development, whereas nymphs feed on the new flush of young leaves and stems. Their piercing mouth parts enable them to feed on phloem tissue, at which point they can take up or deposit the Liberibacter, and then fly to another plant where the feeding continues, and the disease can be spread.

The African and Asian psyllids had different geographical ranges, except for some exceptional islands that serve as a meeting point between the ranges (Fig. 15). Two islands in the Indian Ocean, la Réunion and Mauritius, were just such a meeting point. Extensive study was carried out on la Réunion in the 1970s and 80s to understand the behaviour of the psyllids and test control strategies of HLB.

La Réunion: where CLas and CLaf meet

The island of Réunion lies in the Indian Ocean between Africa and Asia, and aptly is one of the few meeting points of both the African and Asian forms of HLB. Although it is only roughly the size of Dorset (2500 km2), there is a wide variation in temperature and altitude (Figure 16) which provides excellent niches for both the heat-tolerant Asian psyllid and the more sensitive African psyllid.

Agriculture is important on an island with 25% unemployment: 6% of Réunionnais are employed in farming. Citrus groves are relatively small, on average no more than 6 hectares (about 1000 trees), usually intermixed with pineapple or other fruit.

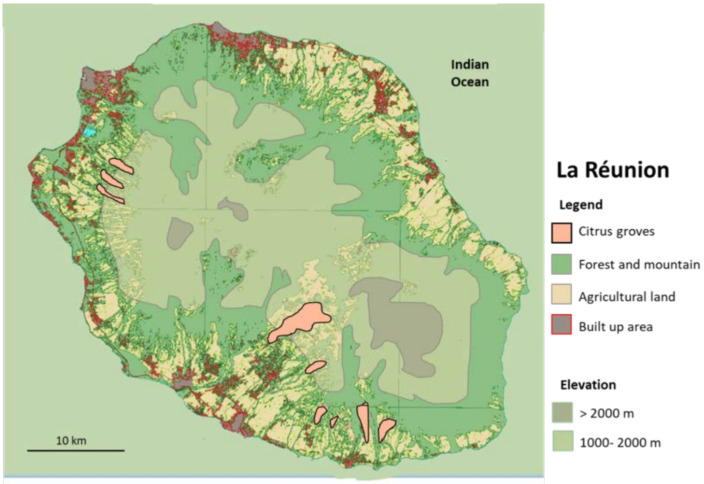

Figure 16: : Reunion, adapted from Dupuy et al., 2019, "Reunion island - 2017, Land cover map (Pleiades). The eastern windward side is wet (9 m rain/year); the leeward side is dry (1 m rain/year)

There are many trees also grown in gardens and parks. The majority of the citrus they produce, mostly mandarins, are sold locally, with only 20% being exported. In fact, about 50% of citrus eaten on the island is imported, which poses a serious problem for those trying to protect Réunion citrus from further HLB infection.

Figure 17 (top, left): The climate on Réunion varies from the flat coastal areas an average 26°C year round (top), to the mountainous interior, usually <18°C, and sometimes with snow (above left). **Figure 18:** (above, right) Citrus stall in neighbouring Mauritius.

HLB symptoms were first recorded in Réunion in 1967 , and psyllids were spotted the following year. In 1970 a sweet orange orchard was planted with 220 healthy trees from Corsica; within ten years almost the entire grove was infected with HLB. Many plant pathologists and entomologists came to Réunion, particularly from mainland France. Réunion became one of the most studied areas for HLB owing to the unusual combination of HLB-forms, but also because its small, isolated position allowed more control of experiments, quarantines, and insect populations.

The heat-tolerant Asian psyllids were mostly found on the leeward side of the island, where the temperatures are higher, and there is less rainfall. The African psyllids were located 500 m or more above sea level in the cool, moist upland areas. Although both forms of psyllid are able to transmit the two different types of Liberibacter, almost all HLB-bacteria found on Réunion matched the range of their usual psyllid.

Mapping the movement of HLB

Plant pathologists and epidemiologists observed the movement of HLB infection on Réunion to understand the pattern and speed of psyllid infection spread. The feeding patterns of psyllids hasten the spread of HLB above and beyond what would be expected from random movement of insects between plants. They found that the psyllids preferred the newly developing leaves (flush) to feed on. The psyllids were attracted to the yellowing colour of HLB-infected leaves, but did not feed on them for long- perhaps a dislike of the texture or feeding experience. The psyllids, having taken up infected sap, would move on to an uninfected leaf to feed for longer, and thereby pass on the bacteria

On Réunion they discovered that psyllids prefer to move to new sites at the edge of groves, working their way along roads, rivers, or farm borders that create an edge-effect. This can increase the spread to new regions or farms. The psyllids themselves often fly up to 75 m per day, which is one limitation to help model the potential spread of HLB. However, in Florida, a hurricane may have carried infected psyllids wholesale across the state, and begun infection in an area that was deemed beyond the reach of the psyllids.

Trying to kill the insect vectors

As a small island, Réunion was very favourably placed to attempt biological control of the HLB vectors. Elimination of HLB would require both removal of infected trees and removal of the psyllid vectors. In the seventies, CIRAD-FLHOR (The Center for International Cooperation in Agricultural Research and Development – Department of Fruit and Horticultural Crops) nursery was established to provide certified clean budstock. After producing >300 000 healthy trees within 25 years, widespread replanting took place.

In parallel with the new planting, in 1974 a controlled release of two types of parasitic wasp took place: *Tamarixia radiata* and *Tamarixia dryii*. These wasps feed on the psyllid nymphs (Figure 19), and thereby limit the vector population size. These parasitic wasps were not native to Réunion, so they themselves did not have any natural predators (hyperparasites) which could have wiped them out before they had brought the psyllid population under control, making them a useful biocontrol to utilise. This two-pronged strategy had astonishing success: by 1980, *T. erytreae* was eradicated from Réunion. A survey in 1995, confirmed that there were no psyllids found in the citrus orchards, and only 0.5% of trees showed any HLB symptoms.

In the eighties, following the almost total eradication of the psyllids, there was a citrus boom, and land used for commercial citrus increased by 300% between 1985 and 1998 (Figure 20). Today, however, citrus plantings have declined to below the 1985 levels.

Figure 19: The Asian citrus parasitic wasp (*Tamarixia radiata*) laying eggs in Asian citrus psyllid nymph

In 2015, HLB had reportedly re-emerged on the south of the island. The French Agricultural Department has sent entomologists and pathologists to understand how the psyllids have overcome the parasites and quarantines in place to ravage the islands citriculture once again. Estelle Roux from a Réunionnais pest control organisation FDGDON hypothesised that tourists illegally bringing fruit and vegetables in their hand luggage could have inadvertently reintroduced psyllids to the island.

Réunion can be seen as an example of a citrus-growing region that was able to control HLB, even if it was not entirely eliminated. The island was helped in this by its geographical isolation from new influxes of psyllids, and the ability of the Réunionnais citrus growers to cooperate on strategies for eliminating infected trees and killing the psyllid vectors. This is in contrast to the struggles faced by Florida, more than 65 times the size of Réunion, and surrounded by shipping and landmasses that can reintroduce psyllids.

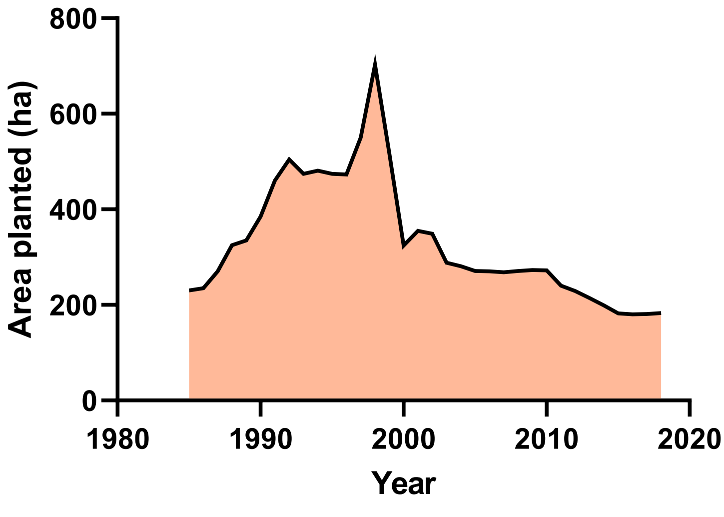

Figure 20: Total area of land used for citrus groves in Réunion from 1985

Huanglongbing in North America: Florida

The arrival of HLB in the Americas

The Americas had been largely insulated from the devastation of HLB until the turn of the 21st century. Florida is the heart of the orange juice industry in the United States. Florida and Brazil together make up over half of global orange juice production. The Asian citrus psyllid (ACP) was first found in Florida in 1998, and had spread throughout the state by 2000, very much a harbinger of doom. HLB was first recorded in Florida in 2005, within 3 years it had spread throughout the state.

However, Florida's citrus industry had already faced a number of challenges since the turn of this century before HLB arrived. Citrus canker remerged in Florida in 1995 and spread throughout the citrus belt. The attempts to eradicate citrus canker were controversial, removing any tree within a ca. 600 m radius of an infected tree. This resulted in mandated removal of more than 2 million trees , and the heartbreak of many growers seeing their family groves destroyed in the space of a year. Florida is a state well used to hurricanes, and yet back-to-back hurricanes in harvest season can devastate a crop, snapping branches, blowing fruit off the trees and damaging any fruit that manages to cling on. 2004-2006 were particularly bad hurricane years for Florida, with Hurricanes Frances and Jeanne hitting the coast two weeks apart.

Into this beleaguered citrus industry, HLB arrived as a straw to break the camel's back. In the 15 years that followed, Florida citrus decreased by ca. 80% (Fig. 21). The decline of the land used for citrus groves and the value of citrus production in Florida is having a serious impact on communities : about 50,000 people work in the citrus industry, and it makes about $7.5 billion per year. In some areas with low tourism, citrus provides over 40% of jobs.

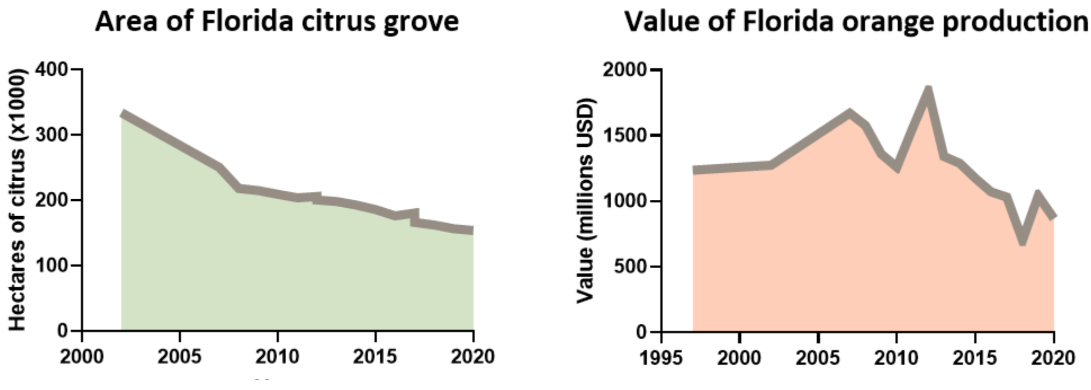

Figure 21: Decline of land used for citrus production (left)) and the value of citrus in Florida (right)

The Florida citrus belt regions, such as Highlands and Indian River counties, are not the Florida with which tourists are familiar. Far from the coast, Highlands citrus representative Ray Royce said that he felt more at home in the farming communities of China than he does driving two hours down the highway to Miami. In Highlands County, 15% of jobs are dependent on the citrus industry, including the processors and the packing houses. Many of the citrus groves in Highlands County have been converted to cattle ranches, or other, more profitable crops. Citrus used to be a common backyard industry, with families making extra money in the harvest season. Today the cost of producing a box of oranges in Florida is almost 3 times as much as it was before HLB. Very few amateur growers remain, as only the big farms can afford the investment of time and money to keep the trees producing against the odds.

Indian River: citrus being squeezed out

Indian River lies on Florida's eastern Treasure Coast, an hour's drive north of Palm Beach (Fig. 22). From the citrus groves you can see the rockets take off from Cape Canaveral. Indian River is believed to be the origin of most of Florida's original citrus groves. Sour citrus trees that were sown by the Spanish in the 1500s, and then cultivated by the English 300 years later, formed the original rootstock for most of the Florida groves. Indian River is unusual among Florida citrus, in that more acres are devoted to grapefruit than oranges. The Indian River trees are forced by the soil structure to grow only lateral roots, which stresses the trees to produce fewer fruit. This, combined with the high rainfall in the region, makes the fruit unusually large and sweet. The main market for Indian River grapefruit is Japan, which will pay an extra $1 per crate for the sweetness.

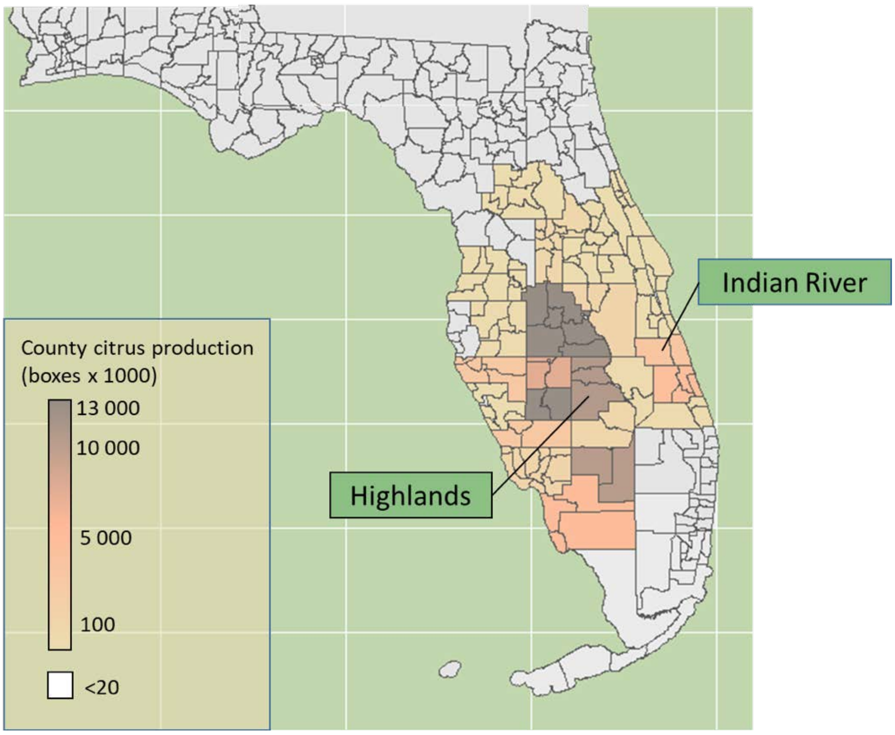

Figure 22: Citrus producing counties in Florida (nass.usda.gov county estimates 2018)

At the time when HLB was arriving in Florida, prices for land on the Treasure Coast were so high that few growers could justify financially continuing with citrus production. In the early 2000s, about 35% of citrus groves were sold to developers. In 2004-2005 many groves in Indian River were abandoned as unprofitable, and the owners waited for land prices to rise before selling. These groves, untended and unmonitored, may have contributed to the rapid spread of HLB prior to its initial detection in 2005.

In order to be financially viable, the packing houses need large volumes of fruit to clean, box and grade. In Indian River county, the volume of fruit is no longer there: the packing houses are merging. In 2017 Hurricane Irma travelled up Florida from the SW one week before grapefruit harvest. The Australian pines planted to protect the groves from the wind were insufficient to prevent 90% of the fruit being blown off the trees. Dan Richey who runs a packing house in Indian River says that HLB is the 'real deal'. In the late nineties 60 million boxes of grapefruit were produced in Florida; today that is down ca. 90% to 4.5 million. Florida citrus was at risk of disappearing altogether unless a concerted effort was made to save it.

Fighting HLB in Florida

Royce from Highland County said that minor crops like citrus are disadvantaged, because there is little incentive for large agri-businesses to invest in research or GM, like they do with wheat and soya. Accordingly, the Citrus Research and Development Foundation (CRDF) was founded in 2009 specifically to support Florida citrus growers. This organisation alone invested about 111 million USD in HLB research. Separately, the USDA has invested upwards of 450 million USD in HLB research since 2009. However, HLB was already present in every county of Florida by this point, so the focus was on elimination or mitigation, and not prevention.

Trying to kill the bacteria

One option considered for controlling HLB in Florida was tackling the Liberibacter directly. This had been tried in Réunion back in 1971: an experimental orchard with severe HLB symptoms provided a test case. On these trees, plant pathologists began testing injection of antibiotics into the trunks of the trees. These antibiotics had a temporary relieving effect of HLB symptoms, but required injection of 2 litres of antibiotic, and tetracycline itself caused some temporary damage to the tree. Fifty years later in Florida, scientists and policy-makers still cannot agree on the risk/benefit balance of using antibiotics to slow the progress of HLB.

Even last year, debate was raging between US government agencies. The US Environment Protection Agency (EPA) has faced opposition from the US public health departments over its authorisation of widespread foliar sprays of two medically important antibiotics: oxytetracycline and streptomycin to control HLB levels in Florida. Objections came from the Food and Drug Administration (FDA) and the Centre for Disease Control and Prevention (CDC) as well as organisations advocating for the environment, public health and consumers. They contended that large-scale use of these antibiotics risked accelerating general antibiotic resistance, posed a threat to farm workers and wildlife, particularly pollinators, and could pollute local drinking water. In March 2019, a petition with more than 45 000 signatures was delivered to the EPA expressing concern about over-use of antibiotics in control of HLB. Later that year, senior US politicians questioned the EPA's decisions to permit antibiotic sprays despite these objections, highlighting research that showed sprayed oxytetracycline was not effective against HLB. However, injection was not a viable option, because it can lead to unacceptable levels of antibiotics accumulating in the fruit.

Trying to kill the insect

With limited success in killing the bacteria in Florida, an easier target was considered to be the vector, the Asian citrus psyllid. One of the many challenges of controlling HLB is the mobility of the vector. The psyllid can travel independently up to 80 m per day. For diseases that seek simply to control the insect population, local sprays of pesticide can be effective. However, the psyllid will have time to feed and pass on the bacteria to the new tree before any foliar insecticide has effect.

Trials in Brazil have shown that insect control works best as a cooperative regional effort. But how to coordinate growers with different sizes of grove, different incomes and different levels of risk aversion to work together? At least 75% of groves need to be sprayed to limit the impact of HLB, and this will include the abandoned groves.

Ariel Singerman from the University of Florida has been applying game theory to try to understand how best to get growers to cooperate on spraying groves to control the Asian psyllid. Public information about risks and benefits of cooperative spraying could encourage cooperation, as well as interaction with local experts and universities. However, Dr Singerman said that 'a change in confidence in science in general' and 'the prevailing politics of the state and the nation' have a negative impact on how likely growers are to cooperate. Currently, there has been limited success in cooperative insecticide sprays and antibiotic applications. The Florida citrus industry broadly is resigned to living with the burden of HLB.

The future of citrus in Florida

Despite the heavy decline in citrus acreage and the soaring costs of production, the remaining citrus growers are broadly optimistic. Indian River growers are confident that their brand will ensure sufficient margins to keep citrus profitable, particularly with the Japanese market. Royce from Highlands Country does not think that this is the end for Florida citrus: those who will continue are replanting groves, often under cover to protect the new trees from psyllids. Farmers are exploring new varieties, and willing to experiment with new rootstock/scion combinations in their own groves. Some have begun planting lemons, the Star Ruby grapefruit, or lemons and limes which are more tolerant of HLB than the current leading citrus varieties. The prevailing mood is cautious optimism from scientists and growers alike. The remaining growers and communities are looking to innovate and diversify to save their industries, some even relishing the possibility of new varieties, and new techniques. There is a resignation that HLB must be controlled, not eradicated.

The Asian citrus psyllid is now being found in gardens on the other seaboard, in California, and every effort is being expended to prevent the spread of HLB into the commercial groves of the Californian hinterland.

Holding back the tide

To date, California has been successful at keeping HLB suppressed, possibly due to slightly more unfavourable conditions for the psyllids, but also learning lessons from the Florida citrus industry. HLB was first detected in California in 2008 in residential trees , although the Asian citrus psyllid had likely been present in California for considerably longer. Non-commercial citrus is much more difficult to control: in Los Angeles ~60% of homes have citrus trees in the garden. California's fruit is aimed at a different market to those in Florida: about 80% of Californian citrus is sold fresh rather than processed. For the fresh market, the appearance of the fruit is important for saleability, so HLB would put even more pressure on growers in California. The focus in California is prevention of establishment of the bacteria, even though the Asian citrus psyllid is already widespread (Fig. 23).

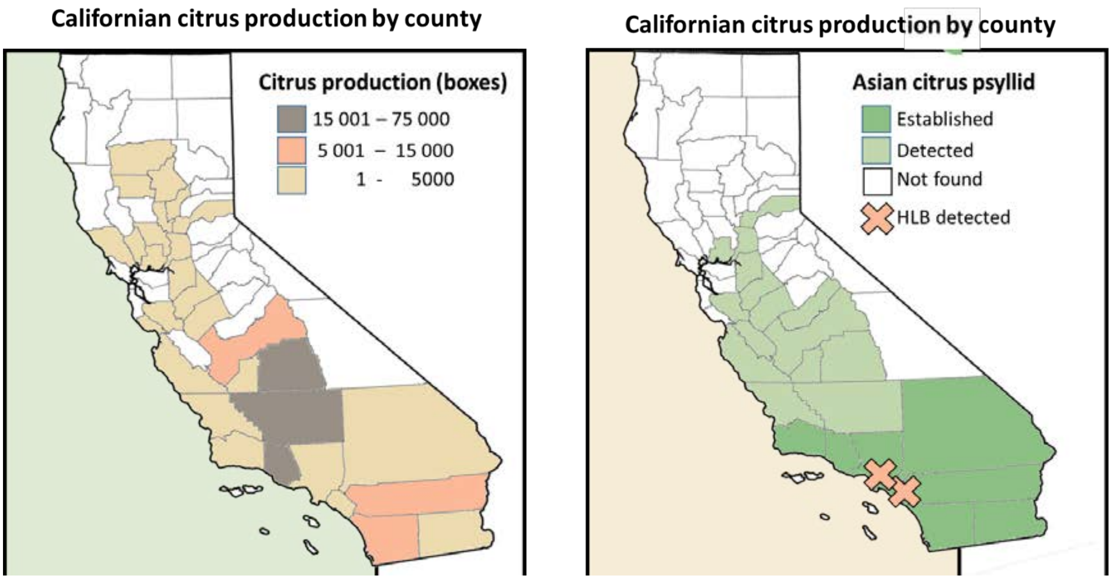

Figure 23: Distribution of citrus production in California (left) and distribution of Asian citrus psyllid with sites where HLB has been detected (right)

Preventing the spread: identifying and removing infected trees

Detecting HLB in a citrus grove is important for limiting spread of the disease. Currently, there is no commercial treatment for HLB, so infected trees are usually removed to avoid spreading the disease. The presence of the psyllids themselves is not enough to show that HLB will be present. Uninfected psyllids are present in many regions, and cause feeding damage relative to their numbers, but not the wholesale destruction caused by CLas-carrying psyllids.

Problems with early detection of HLB

Originally, testing for HLB was purely an assessment of ear symptoms: yellowing of leaves, and discolouration and malformation of fruit. However, a tree can be infected with HLB for two or more years without showing noticeable signs of disease , and can therefore be a node of infection for the surrounding area well before any preventative action can be taken. Significant yield loss can also occur independent of the symptom severity; this may be due to initial root infection. The underdeveloped and fibrous root symptoms of HLB were initially thought to be a secondary symptom of Liberibacter infection.

However it has recently been found that CLas is detected in the roots prior to leaves in infected citrus trees, with root symptoms becoming apparent before foliar symptoms. Symptoms can fluctuate throughout the year, with diagnosis more difficult in the warmer seasons. However as citrus is an evergreen perennial, HLB can continue to develop until trees become so severely infected and unproductive that they are no longer economically viable. Therefore, a focus of research in recent years has been early and reliable detection of HLB-infected trees.

Citrus trees, like all flowering plants, have tubes called phloem which carry sugary sap made in the leaves throughout the plant, particularly to roots and areas of new growth. Liberibacter collect in the phloem, but are not evenly spread within the phloem tissue, nor indeed throughout the whole plant. This makes sampling trees to test for HLB very difficult: it is very easy to get a false negative, even when checking for the presence of bacterial DNA.

New methods are being developed to detect early signs of HLB. Citrus regions under threat from neighbouring HLB-suffering areas, such as California and Australia, are particularly keen to develop early detection techniques. There are trials in California of an innovative method to detect early-stages of HLB, before any symptoms even develop.

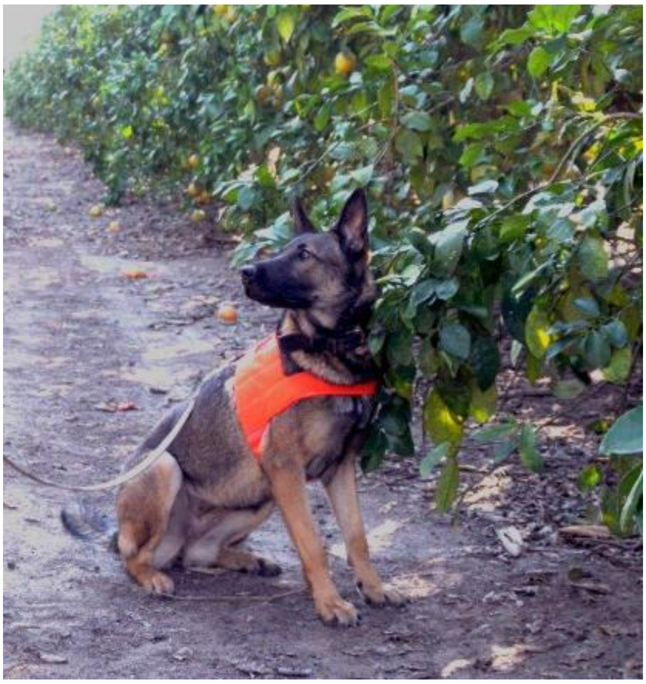

Figure 24: Canine detection of HLB (Gottwald et al., 2020)

The Gottwald lab from the US Department of Agriculture (USDA) has found that sniffer dogs can detect HLB 32 days after infection with HLB (Fig. 24) , compared to the most common method of detection: visual assessment followed by measuring the levels of bacterial DNA in the tree (PCR). PCR can provide false negatives for up to 32 months on a tree due to the uneven distribution of the bacteria in the tree, and the low levels found at the early stages of infection. The dogs patrol the lines of trees, and alert their handlers to the presence of HLB-infection cues by sitting down next to the tree.

The future of Huanglongbing

Citrus industries under threat: Mediterranean and Australia

In the last one hundred years, the spread of HLB has been monitored across Asia and Africa, into South and North America. The understanding of the disease that plant pathologists and entomologists have developed over the last century have been unable to prevent devastating infection levels in Florida, and the encroachment on Brazilian and Californian groves. Australia and the Mediterranean citrus growing areas are braced with HLB on their doorsteps: Papua New Guinea and the Canary Islands respectively, and southern Africa has new species of psyllid and Liberibacter encroaching (Fig. 25).

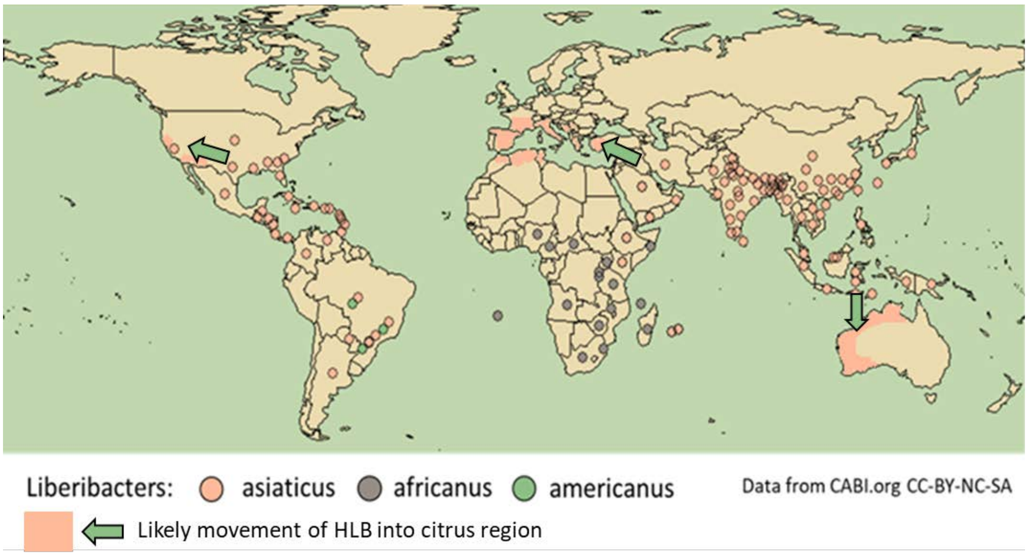

Figure 25: Citrus growing regions around the world that are under threat of HLB

Where will HLB spread next?

Modelling based on current climate conditions suggests that Western Australia is particularly suitable for both bacteria and vectors in terms of climate (Figure 25). An insufficient level of rainfall in the wettest seasons makes the Mediterranean basin and the Central Valley of California less appealing to the vector. This climatic deterrent could, however, be counteracted by irrigation and climate change. And longer-term modelling of pests and diseases has to take into account the projected changes in climate we anticipate in the next 100 years. An increase in average temperature globally could impact on the psyllids rate of reproduction, their range and the variety of hosts they feed on.

The Mediterranean

The Mediterranean region accounts for <10% of world citrus production , and has been largely untouched by HLB. As a result, total citrus fruit production in the EU has remained fairly constant: decreasing by only ~2% in the last ten years , compared to a 67% decrease in Florida. The African psyllid, *T. erytreae*, was detected on the islands of Madeira (1994) and the Canary Islands (2002), and as of 2014 has spread to the west of the Iberian Peninsula. However, the presence of HLB in Europe has not yet been detected.

The Mediterranean basin is seen as a particularly vulnerable area for invasion by new pests and diseases owing to its higher than average climate change, high biodiversity, and the almost uncontrollable quantity of points of entry by land, sea and air. As such, the European Union has set up an action group "LIFE Vida for Citrus" to identify HLB-resistant cultivars that grow well in Southern Europe, and test early HLB-detection techniques (Fig. 26). Life for Citrus has carried out an experimental release of biological control parasites *Tamarixia dryii* in Galicia, northern Spain, where the African psyllid (*T. erytreae*) has been identified in order to eliminate the psyllids before the Liberibacter is found on the European mainland.

Figure 26: Orange groves under threat in Andalucía, Southern Spain (left) and propagation of citrus plants in preparation for HLB-resistant citrus trials in Andalucía (right)

There is also a consortium of EU universities (PRE-HLB) coordinating on preventative measures to keep HLB at bay. In 2019, Candidatus Liberibacter spp. joined the priority pest list of the EU, which will force member states to monitor and protect against the HLB causative agent. The European Union places restrictions on the types and extent of insecticide and antibiotic use, so biological controls and preventative measures are preferred avenues to explore.

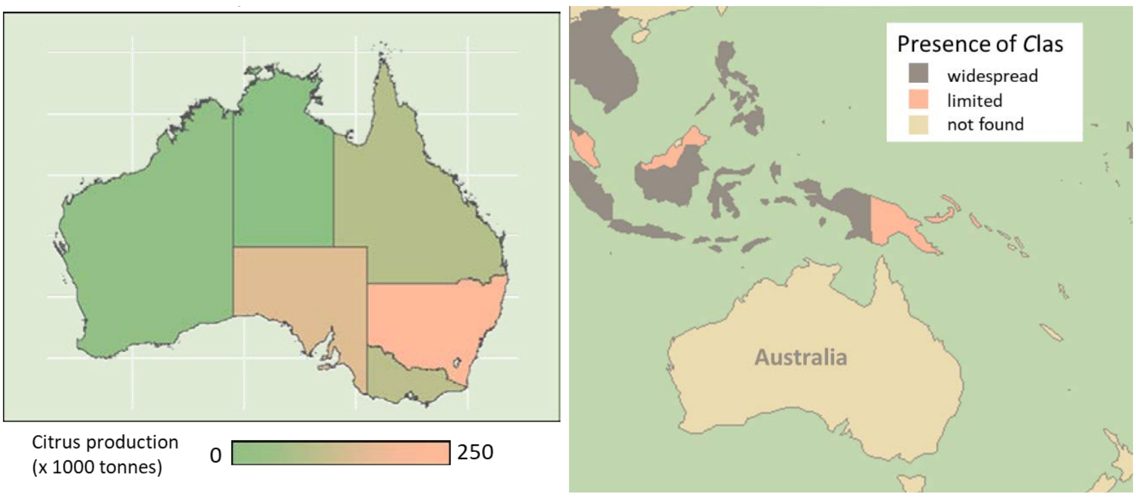

Figure 27: Citrus production in Australia per state (left) and presence of Candidatus Liberibacter asiaticus (B)

Australia

Australia is not a large citrus producer (<0.5% global citrus), but orange exports alone are worth >225 million USD. The main citrus growing areas in the south of the country (see above) are not ideal breeding grounds for the psyllids, but the smaller scale groves in Western Australia are both geographically close to HLB infected countries (Figure 27), and climatically suitable to HLB.

Figure 28: Mock orange (*Murraya paniculate*) is one of several ornamental host plants that pose a threat to HLB spread. They are asymptomatic hosts, widespread in Australia

Australia has historically had strict regulations in place for restricting entry of plant material, and so far, these restrictions have prevented any outbreaks of HLB. The Asian psyllid has been found on budwood, lime leaves and other undeclared citrus material that has been confiscated at airports; there is also a risk that the psyllid could arrive in northern Australia from South-east Asia via jet streams or cyclonic winds. Ornamental host plants, such as mock orange, *Murraya paniculata* (Fig. 28), also pose a threat, as they do not show symptoms, and are widely planted throughout Australia. Similar to the Mediterranean citrus industry, Australian citrus producers and government are investing in preventative measures and research.

South Africa

South Africa has already dealt with one wave of HLB. By the mid-1970s, there were an estimated 4 million HLB-infected citrus trees with the heat-sensitive CLaf. However, as in Réunion, HLB was brought under control with tight legislation. Growers could only plant healthy, certified material and movement of planting material between infected and HLB-free areas was limited, in addition to chemical controls. There remained some CLaf in the cooler citrus-growing regions , but the impact on the citrus industry was mitigated and planted acreage continued to increase. However, an impending threat to South Africa is the arrival of the Asian citrus psyllid, *D. citri*.

Previously the ranges of the Asian and African citrus psyllids were quite distinct, with the exception of Réunion and Mauritius. However, *D. citri*, has been detected over the last few years in Tanzania, Kenya most recently just north of Mozambique although they were reportedly free of the bacteria. Equally worrying, the African psyllid *T. erytreae* was detected carrying CLas in Ethiopia in 2010 and 2017. With the increasing spread along the east coast of Africa the CEO of Citrus Research International, Dr Vaughan Hattingh, has warned that it is only a matter of time before the Asian psyllid vector and in turn the CLas disease arrives in South Africa. Due to its increase in heat tolerance, this poses a new threat to the South African citrus industry.

With increasing uncertainty about the future of HLB-sensitive oranges, the acreage of orange groves is decreasing in South Africa; the more HLB-tolerant lemons and limes are becoming a more popular crop (Figure 29).

What comes next?

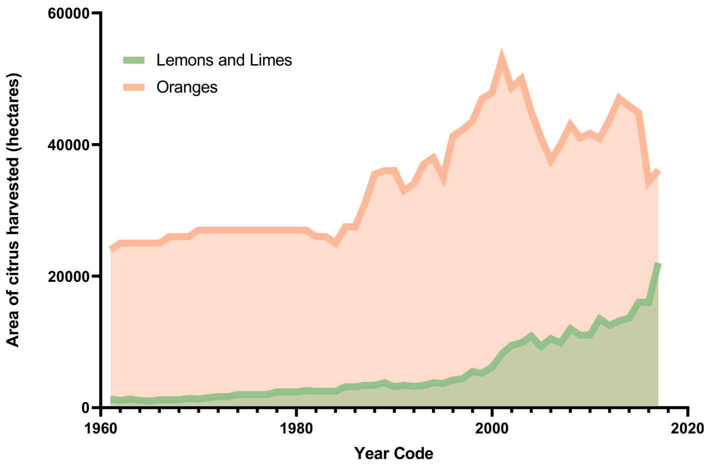

Figure 29: Area of citrus plants harvested in South Africa since 1960

There are many aspects of HLB that make it a difficult disease to control: the multiple modes of spread, the mobile vectors, the difficulty in detection, the variety of potential cultivated and wild host plants, and the lack of known remedy. Certainly citrus farmers in Florida are resigned to living with, and attempting to limit, HLB, rather than eradicating the disease. However, there are some bright spots on the horizon: quarantine successes, development of resistant citrus varieties and potential treatments to eliminate the bacteria from trees

Solutions in plant breeding

Scientists have been searching for varieties of citrus with greater resistance to HLB since the early 20th century. All of the citrus crops are susceptible to HLB, to a greater or lesser degree. Unfortunately, the world's most popular citrus fruit, sweet oranges, are the most sensitive to the disease, developing symptoms very quickly (Figure 30). The most tolerant varieties of citrus include Persian lime (usual variety found in UK supermarkets) and the small Eureka lemons, which can be infected with HLB, yet continue to grow and fruit quite normally.

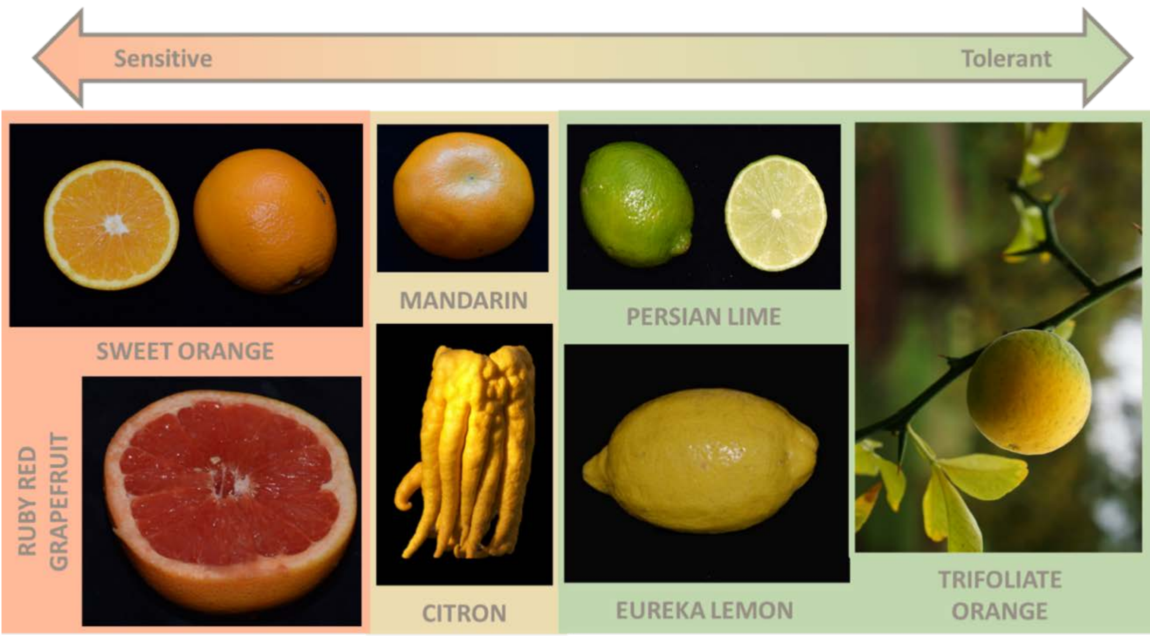

Figure 30: Sensitivity of citrus varieties to HLB

The close relative to citrus, trifoliate orange (see above) *Poncirus trifoliata*, can tolerate high levels of HLB without significant symptoms. In this, trifoliate orange presents both a threat and an opportunity for commercial citrus. A threat, because it is widely planted in gardens, and can serve as an asymptomatic reservoir of the disease; an opportunity because it presents possibilities for plant breeding and use as a rootstock. The trifoliate orange genome has recently been sequenced to aid these plant breeding efforts.

Potential new treatment

In July 2020, a potential breakthrough for HLB treatment was announced by the lab of Professor Hailing Jin at the University of California, Riverside. For about five years, Prof Lin's lab had been investigating compounds made by Australian finger limes, which are naturally resistant to HLB infection. They managed to identify a peptide that, if applied by spray or injection on an infected tree, can clear symptoms and regenerate growth after a couple of months; it also has potential to protect uninfected trees. The peptide treatment is now going to trials in Florida groves to test it in real –world scenarios. Florida researchers and growers are cautiously optimistic about the treatment, holding judgement until the price and efficacy become clear.

Concluding remarks

The spread of HLB over the last century has been slow but almost inexorable. From its starting point in the home of citrus in East Asia, the disease has come to infect the groves of the largest citrus producers in the world: China, Brazil and the USA, and is poised on the doorstep of Europe and Australia.

There are many aspects of HLB that have thwarted the efforts of plant pathologists and growers to understand or control it. It has been difficult to disentangle the symptoms from those of so many afflictions of citrus trees, both biotic and abiotic. Over the years, HLB symptoms have been variously attributed to waterlogging, nutrient stress, nematodes, a virus, a mycoplasma and finally the unculturable Candidatus Liberibacter spp.

The bacteria cannot be grown outside of a plant, making it difficult to use in experiments, or even see under a microscope. The disease remains asymptomatic for years, and is unevenly distributed throughout the tree, so testing for the presence of HLB is unpredictable. And it has resisted most attempts to date to treat it. It has taken advantage of both human horticulture and insect feeding to spread across oceans and continents. It is small wonder that it has wrecked so much havoc on the global citrus industry.

There is no doubt that the impact of HLB has been severe on the individuals and communities that rely on citrus farming for their livelihood. In many HLB-infected regions, growing citrus is not worth the cost, uncertainty, hassle and heartbreak. HLB has continued its relentless progress despite the best efforts of growers, governments and scientists. There is no doubt that a century of plant pathologists from every continent have worked indefatigably to bring to light first the cause of the symptoms, and latterly potential solutions.

Indeed, cautious optimism remains: some quarantine and biological control efforts have been successful. The long process of fruit breeding may yet produce the HLB-resistant sweet orange that the consumer loves. And the mooted peptide treatment that could cure HLB-infected trees could bring hope to citrus farmers in Florida, South Africa and China alike.

Further reading

Some excellent review articles on HLB

Bové J. (2006). Huanglongbing: A destructive, newly emerging, century-old disease of citrus, *Journal of Plant Pathology,* **88**: 7-37

da Graça JV. *et al.* (2016). Huanglongbing: An overview of a complex pathosystem ravaging the world's citrus. *Journal of Integrative Plant Biology*, **58**: 373–387

Gottwald TR (2010). Current epidemiological understanding of citrus huanglongbing, *Annual Review of Phytopathology*, **48**: 119-139.

Key studies in understanding HLB

Lin KH (1956). The Citrus Huang Lung Bin (Greening) disease in China. *Acta Phytopathologica Sinica*, **2**: 14–38.

McClean PCJ, Oberholzer APD (1965). Greening disease of sweet orange: evidence that it is caused by a transmissible virus, *South Africa Journal of Agricultural Science.* **8**: 253 - 276

Saglio *et al.* (1971) Isolement, culture et observation au microscope électronique des structures de type mycoplasme associées à la maladie du Stubborn des agrumes et leur comparaison avec les structures observés dans le cas de la maladie du greening des agrumes. *Physiol Veg,* **9**: 569–582

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Roots: CC BY 4.0 Dr Evan Johnson, University of Florida from Nehala and Kiliny (2020), Revisiting the Complex Pathosystem of Huanglongbing: Deciphering the Role of Citrus Metabolites in Symptom Development, metabolites.

Figure 5:

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Figure 8:

Photo of Professor Lin from hnr.scau.edu.cn (accessed 27/11/2020)

https://hnr.scau.edu.cn/2019/1108/c8272a203076/page.htm

Figure 2:

Adult African citrus psyllid (Trioza erytreae): CBG Photography Group, Centre for Biodiversity GenomicsBIOUG25714-F07 CC-BY-NC

African citrus psyllid nymph: Peter Stephen, Citrus Research International, Bugwood.org. Image No. 5137030 CC-BY-NC

Adult Asian citrus psyllid: Pest and Diseases Image Library , Bugwood.org Image No. 5312097. CC-BY-NC

Asian citrus psyllid nymph: David Hall, USDA Agricultural Research Service, Bugwood.org Image No. 500608. CC-BY-NC

Figure 17:

"Petit marché de Saint-Denis de La Réunion" by Philippe Vieux-Jeanton © CC0 1.0. https://www.flickr.com/photos/11234074@N05/8672930688 (Last accessed 26/10/2020)

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