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# The Asian Citrus Greening Disease (Huanglongbing):

## Evidence Note on Invasiveness and Potential Economic Impacts for East Africa

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**Cover photo**: Citrus tree with severe dieback, a typical symptom of citrus greening disease, Kwale County, Kenya. Credit: Ivan Rwomushana, CABI.

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# Abstract

Huanglongbing (HLB), also known as citrus greening disease, is one of the most devastating pathogens of citrus worldwide and is caused by closely-related species of systemic *Candidatus* bacteria. Vectored by the Asian citrus psyllid (ACP), *Diaphorina citri*, the heat-tolerant, Asian form of the disease, *Candidatus* Liberibacter asiaticus (*C*Las) is the most serious and widespread. In East Africa, the heat-sensitive form of the disease is vectored by the African citrus triozid (ACT) *Trioza erytreae* and remains the most prevalent disease, particularly in the cooler, mid to high altitude areas. Since 2010 however, *C*Las which can also be transmitted by *T. erytreae*, has been spreading in the African continent and significantly, the Asian HLB vector has also been detected in Tanzania and Kenya. There is now a clear and present threat of HLB to the citrus industry in Africa, including the previously sustainable warmer citrus producing regions of East Africa. Modelling of environmental suitability suggests that without preventative measures *C*Las could establish widely in Africa, with potential hotspots in Central and South-eastern Africa, incurring substantial economic losses. The following evidence note reviews the global literature on the HLB pathogen-vector complex, highlighting potential risks and estimating economic impact for Africa. A synthesis of recommendations for biosecurity preparedness, surveillance and management options is outlined to inform decision makers and growers.

# Executive summary

## Background

Asian citrus greening disease or huanglongbing (HLB), caused by the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), is a devastating disease of citrus trees which has spread globally via trade from its origins in Asia, posing a huge threat and economic burden to nearly all citrus producing regions of the world. Global interventions to prevent the spread of HLB and reduce its impact have been, for the most part, ineffective. The primary vector for this disease, *Diaphorina citri* (Asian citrus psyllid, or ACP) is present in East Africa and the recent detection of HLB in Kenya and Tanzania brings into sharp focus the need for coordinated action to contain and/or eradicate the disease before it spreads and becomes ubiquitous in the region. This evidence note therefore provides information on the key facts about HLB, including the losses currently experienced and estimations of potential further losses if HLB continues to spread and becomes endemic throughout citrus-growing regions of Africa. A summary of the research and development on control measures is provided, as well as a synthesis of recommendations for management of HLB and its vectors. This information will be useful for a wide range of stakeholders, including researchers, policy makers, donors and other high-level decision makers.

## Strategic importance of citrus

From an economic point of view, citrus fruits rank first in terms of world fruit production and international trade value. The world production of citrus for 2018 was estimated at 139 million tonnes (cultivated in over 140 countries around the world), the major producers being China, Brazil, the USA and countries bordering the Mediterranean. Average citrus production for all of Eastern Africa was 1.2 million tonnes in 2018. Citrus is an important domestic crop throughout East Africa, where production is mainly by smallholders. The yields of smallholder farmers in East Africa often do not exceed 4–10 t/ha, while the crop has the potential of producing up to 50–70 t/ha in countries practicing integrated pest management (IPM). In East Africa, Tanzania has overtaken Kenya as the largest citrus producer after Kenya was severely impacted by the African form of the greening disease (African citrus greening disease, AfCGD) transmitted by the African citrus psyllid (ACT).

## Understanding African and Asian citrus greening (Huanglongbing) and their vectors

Citrus greening disease is associated with three Gram-negative bacteria: *Candidatus* Liberibacter asiaticus (*C*Las), *Candidatus* Liberibacter africanus (*C*Laf), and *Candidatus* Liberibacter americanus (*C*Lam). The liberibacters are native to particular geographical zones but in modern times have spread, along with their psyllid vectors, to new areas in association with the development and extension of susceptible crops. *C*Las, vectored by the temperature tolerant Asian citrus psyllid, *D. citri,* is associated with Asian HLB and both it and its vector are the most prevalent and devastating pathosystem, having spread worldwide from China. The African form *C*Laf, which shows symptoms under somewhat moist and cool conditions and at higher elevations, is transmitted by the African citrus psyllid, *Trioza erytreae* and causes AfCGD. Whilst it is considered less debilitating than *C*Las-associated HLB, it is a widespread and persistent problem in Africa, where a number of new lineages of the species have been reported.

## Huanglongbing symptoms and crop damage

The HLB bacteria can infect most citrus cultivars, species, and hybrids, as well as some citrus relatives. The first symptom of HLB is usually the appearance of a yellow flush of growth on an infected tree, hence the name "huanglongbing" which translates from the Chinese as "yellow dragon" or "yellow shoot disease". Progressive yellowing and thinning of the entire canopy follows, leaves turn paleyellow and display characteristic blotchy mottling or vein yellowing, point upwards and are reduced in size. In the early stages of infection, diagnosis can be difficult as trees remain symptomless, however chronically infected trees become sparsely foliated and show extensive twig dieback and eventually, premature death, which can occur several months to years after infection. The fruits, which are abnormally bitter in taste, are often small, lopsided with aborted dark seeds, and remain green in part (hence the origin of the name greening) and drop prematurely reducing yields. Severe symptoms in trees have been observed one to five years after onset of the first symptoms, depending on the age of the tree at the time of infection, but also on the number of infections per tree, which are often multiple. The disease's rapid progress, combined with the impact on yield and quality of fruits means that affected orchards can lose their economic viability within seven to ten years of planting.

## Current spread and distribution of Huanglongbing in East Africa

Huanglongbing was first detected in Ethiopia in 2010 and continues to spread within the country including to regions distant from the first detections and at high altitudes. Assays of *T. erytreae* (ACT) collected in the field have confirmed infection with *C*Las raising the prospect of HLB proliferation aided by a novel additional vector. Recent reports have now also confirmed the presence of the primary HLB vector, *D. citri* in Ethiopia including at relatively high altitudes, with imminent spread predicted. *D. citri* has also been reported from sites across the south of Kenya to the coast and in the north-east of Tanzania, an important citrus-producing region, with spread ongoing. The recent discovery of *C*Las in the coastal region of Kenya close to the border with Tanzania, particularly in the presence of its increasingly widespread primary vector is understandably a cause of great concern for citrus producers in the area. HLB and its vector are yet to be confirmed in Uganda, but it is clear that citrus production across Eastern Africa is under severe threat from this destructive disease. The recent arrival of *D. citri* in Nigeria, a major producer of citrus, raises the prospect of HLB proliferation in West Africa, a region predicted to be highly suitable to the disease and its primary vector.

## Economic impacts of Huanglongbing in East Africa

The yield losses to Asian citrus greening, once widely established, can be expected to be significant and a potential threat to ongoing citrus production for resource-poor growers. At this early stage of invasion, however, production losses in citrus due to HLB in Eastern Africa are likely to be limited and have received minimal analysis in the literature. As infected plants age and symptoms are increasingly expressed, and the disease spreads via its vector(s) to infect citrus more extensively across the region, as is probable, the losses to citriculture could be very significant. Producers in hotter lowland areas who are currently spared losses to the less heat-tolerant African citrus greening disease could now face major impacts from the more damaging and heat-tolerant Asian citrus greening. The majority of citrus in the region is now at potential risk from one or both of these diseases. Extrapolating HLB production losses equivalent to those reported for Florida, the estimated annual value of lost production in four East African countries (Ethiopia, Kenya, Tanzania and Uganda) ranges from US$ 21.3 to 63.8 million within five to ten years. Over a longer timeframe (ten to fifteen years), estimated annual production losses range from US$ 63.8 to 127.6 for these countries. Economic losses could be greater still for major sub-Saharan African citrus producers such as Nigeria and South Africa should

HLB arrive, underlining the pressing need for rigorous and practicable action plans for vulnerable citrus-producing regions.

## Huanglongbing and trade

HLB is primarily a vector-borne disease but the risk of local and regional spread is further increased by movement of frequently traded plant products from infected areas. Trade of infected host citrus material (budwood, grafted trees, rootstock seedlings) and alternative hosts such *Murraya koenigii* in the Rutaceae family, are recognized pathways for HLB introduction and can also carry Asian citrus psyllid eggs and/or nymphs over long distances. Increasing regional trade, reservoir host plants and similar climatic patterns are likely to contribute to spread of the disease. The arrival of *D. citri* in mainland Africa presents a serious threat to African citriculture since citrus production at all altitudes would be at risk, potentially resulting in the industry becoming economically non-viable within a few years. Due to the severity of HLB, EPPO recommends the prohibition of importation of citrus plants for planting and cut branches or buds of citrus from areas or countries where HLB or either of its vectors are present. Both *T. erytreae* and *D. citri* are recommended for regulation as quarantine pests in the European Union, whereby exporting countries may be required to apply mandatory phytosanitary procedures, which results in extra cost to exporters and the National Plant Protection Organizations. At present, none of the bacteria associated with HLB have been found in the EPPO region, although one of its vectors, *T. erytreae*, is present in Spain and Portugal. In South Africa, the National Plant Protection Organization is developing an early warning system in collaboration with the citrus industry as part of the South African Emergency Plant Pest Response Plan and has alerted the agricultural community, importers, researchers and smallholders to the risks of HLB to the entire continent.

## Management of Huanglongbing

To date, there has been a decline in all commercial citrus industries that have faced this disease and with adequate control still lacking, most of the citrus-producing countries remain under threat. Disease management is complicated by long incubation periods and difficulties of diagnosis. The first line of defence for HLB has always been quarantine measures to ensure the bacteria is prevented from entry but once introduced, psyllid management is critical to slow establishment and spread, and this invariably involves a persistent spray regime to maintain low vector populations. In the absence of sustainable curative treatments or resistant citrus lines, countries not yet severely affected by HLB have demonstrated some benefit by adopting rigorous and coordinated approaches to management. The "3-pronged" preventive measures revolve around the use of disease-free nursery stock (produced in insect-proof nurseries), area-wide suppression of the vector, *D. citri*, and eradication of symptomatic trees to reduce or eliminate inoculum sources. However, these efforts are often undermined by the ubiquitous presence of disease reservoirs from citrus trees across the region, in commercial orchards and household gardens, as well as alternative hosts such as ornamental *Murraya* species, where subtle symptoms may go undetected. Whilst the use of insecticides can form an important and cost-effective component of HLB management, their application needs to be judicious and well timed since overapplication of pesticides not only increases costs but also leads to resistance development and has negative environmental and human health implications. Many alternatives to chemical control such as thermotherapy, shoot-tip grafting, antibiotic treatment, nutritional sprays and pruning have provided some short-term relief but proved impractical options long term. Furthermore, these can even undermine the effectiveness of broader mitigation measures, by allowing sources of inoculum to persist. Classical biocontrol using parasitoid wasps has been successful in small, non-continental land masses but is unlikely to reduce *C*Las transmission sufficiently to mitigate against HLB. Substantial research investments are made worldwide into HLB, its vector and citrus breeding, and the advances of modern biology and chemistry provide some hope that new research directions may offer ways to combat this devastating disease.

## Huanglongbing advice and information

Huanglongbing is still a new disease in Kenya, and consequently not much information is being disseminated on the proper identification of the disease and how to differentiate it from African citrus greening disease, as well as its vector. ICIPE has recently completed a project on citrus pests and diseases and one of the next steps suggested was to compile and document all the available information on the efficacy of different management options of HLB and its vector, learning from experiences in the USA, China and South Africa. Consequently, awareness-raising among the farming communities, extension and quarantine personnel is required. This could be through mass media, ICT, training and communication materials in local languages, amongst other methods. In terms of control, HLB does not lend itself to many of the common methods employed for most pests as the disease is often detected late when the citrus tree starts showing symptoms. Therefore, any recommendations communicated to the farmer would need to be efficacious, safe, sustainable, practical, available and affordable.

## Recommendations

The following key recommendations are provided for the sustainable management of HLB in Africa.

**High-level policy makers:**

- Recognise the magnitude of the HLB threat (present and potential) and make policy decisions, backed by available economic estimates and science-based evidence
- Lobby for budgetary allocation to facilitate an immediate, official national response backed by policy and legislation to enable coordinated (potentially mandatory) contingency action to curb the spread of the disease at the earliest opportunity
- Aim to create preparedness action plans across the East African Community and harmonize with established steering committees across the continent (e.g. South Africa's Emergency Plant Pest Response strategy and HLB action plan) to mitigate and contain the disease

**Regulators:**

- Strengthen the content and enforcement of phytosanitary regulations on movement of citrus plants and alternate hosts
- Fast-track the process of testing, validating and registering products for the control of the HLB vector

**Researchers:**

- Carry out surveys to identify local natural enemies that can be used in augmentative and conservation biocontrol

- Test locally-available biopesticides and other natural materials and produce formulations that can maximise the toxic effects on ACP whilst limiting the side effects on beneficials

**Advisory services:**

- Expand extension efforts to facilitate and coordinate judicious control of the psyllid vector if detected, promoting IPM and low-risk options for management, as well as rapid removal of infected trees in commercial, smallholder and abandoned citrus groves

- Develop an array of cost-effective, clear and harmonized monitoring and impact assessment protocols to be followed for HLB studies

**Smallholder farmers:**

- Engage in early warning surveillance, with ACP scouting and trapping as part of routine insect pest scouting programmes

- Treatment and removal of infected trees as a priority and replacement with healthy trees, with added protection from psyllid ingress in hotspot areas

**Commercial farmers:**

- Citrus nurseries are encouraged to proceed with changing over to citrus tree production in insect secure structures

- Commercial growers need compliance agreements to ensure production of certified disease-free material and implementation of phytosanitary protocols for movement and elimination of inoculum

# Acronyms

| | |
|---|---|
| ACP | Asian citrus psyllid |
| ACT | African citrus triozid |
| AfCGD | African citrus greening disease |
| ACGD | Asian citrus greening disease |
| AEZ | Agro-ecological zone |
| CABI | CAB International (Centre for Agriculture & Biosciences International) |
| CHMA | Citrus Health Management Areas |
| *C*Laf | *Candidatus* Liberibacter africanus |
| *C*Lam | *Candidatus* Liberibacter americanus |
| *C*Las | *Candidatus* Liberibacter asiaticus |
| CRI | Citrus Research Institute |
| CRDF | Citrus Research and Development Fund |
| DALRRD | Department of Agriculture, Agrarian Reform and Rural Development |
| EIL | Economic injury level |
| EPPO | European Plant Protection Organisation |
| ETL | Economic threshold level |
| FAOSTAT | Food and Agriculture Organisation Statistical Database |
| HLB | Huanglongbing |
| HMO | Horticultural mineral oil |
| ICIPE | International Centre of Insect Physiology and Ecology |
| IGR | Insect growth regulator |
| IPM | Integrated pest management |
| NPPOZA | The National Plant Protection Organization of South Africa |
| PMDG | Pest Management Decision Guide |
| PPE | Personal protective equipment |
| SADC | Southern African developing countries |
| USDA | United States Department of Agriculture |

# Introduction

Citrus is one of the world's major fruit crops, recognized for contributing to food and nutritional security, and ranks first in the global fruit trade. Indeed, citrus has remained the most sought-after global fruit during the Covid-19 crisis, as people seek to boost their immune system by increasing vitamin C intake. However, several biotic and abiotic factors are responsible for decreasing yields and substantial economic losses worldwide. Among these, the insect-transmitted, phloem limited bacterial disease, Huanglongbing (HLB), also known as citrus greening disease, is one of the most ravaging pathogens of all commercial citrus varieties, for which an effective cure remains elusive. Three closely-related species of Liberibacter are known to cause the symptoms of greening disease: *Candidatus* Liberibacter asiaticus (*C*Las), *Candidatus* Liberibacter americanus (*C*Lam), and *Candidatus* Liberibacter africanus (*C*Laf). The heat-tolerant, Asian form of the disease, transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is the most devastating and widespread. In East Africa, the heat-sensitive form of the disease caused by *C*Laf (African citrus greening didease (AfCGD) is vectored by *Trioza erytreae* (Hemiptera: Triozidae), also known as the African citrus triozid (ACT) (Aubert, 1987) and remains the most prevalent disease, particularly in the cooler, mid to high altitude areas, causing yield losses of 25–100% in Kenya and Tanzania (Richard *et al*., 2018).

In the last decade, *C*Las and its heat-tolerant insect vector have been spreading to the African mainland and there is now a clear and present threat of HLB to the citrus industry in Africa, including the previously sustainable warmer citrus-producing regions of East Africa.

In view of the threat posed by this pest complex, this evidence note aims to review the comprehensive literature on HLB and its management from global experiences, and consolidate the relevant research to provide recommendations for decision makers in Africa responsible for the response to the insect vector and disease, as well as for external organizations seeking to assist in management. This information will be useful for decision makers to prioritize investment and interventions in responding to the growing threat. This evidence note is structured into five sections as follows:

- **Section 1** reviews citrus production trends, the citrus greening problem, current distribution of the pest and vector and its environmental suitability.
- **Section 2** reviews the impacts of citrus greening in other affected regions and make informed predictions on citrus yield losses and impact on trade in East Africa.
- **Section 3** provides information on ongoing and recent research on control methods, highlighting the range of options using other countries as examples.
- **Sections 4 and 5** conclude with some sources of information and final recommendations for key stakeholder groups in Kenya (with relevance to neighbouring African countries).

# 1 Citrus production and the citrus greening problem

## 1.1 Commodity context

The various species of citrus (Rutaceae) are all believed to be native to the subtropical and tropical regions of Asia and the Malay Archipelago, and to have spread from there to all other continents where they have been cultivated throughout the ages. As many as 300 species have been described, though the group presents taxonomic challenges due to the marked sexual compatibility between the different species, which has generated hundreds of varieties. The term citrus fruits includes different types of fruits and products. Although oranges are the major fruit in the citrus fruits group, accounting for about 70% of citrus output, the group also includes small citrus fruits such as tangerines, mandarins, clementines and satsumas, as well as lemons, limes and grapefruits. Today, an exceptional array of varieties of citrus trees have evolved and been developed through natural evolution and artificial selection. Wu *et al.* (2018) suggest citrus diversified during the late Miocene epoch through a rapid Southeast Asian radiation that correlates with a marked weakening of the monsoons.

From an economic point of view, citrus fruits rank first in terms of world fruit production and international trade value. The world production of citrus for 2018 was estimated at 139 million tonnes (cultivated in over 140 countries around the world), the major producers being China, Brazil, the USA and countries bordering the Mediterranean (Fig. 1). Nigeria is the largest citrus producer in Africa, producing around 4 million tonnes (FAOSTAT, 2019), with acreage and production having dramatically increased thanks to suitable ecological and climatic conditions for production (Oke *et al*., 2020). Eastern Africa accounts for around 137,000 tonnes (FAOSTAT, 2019).

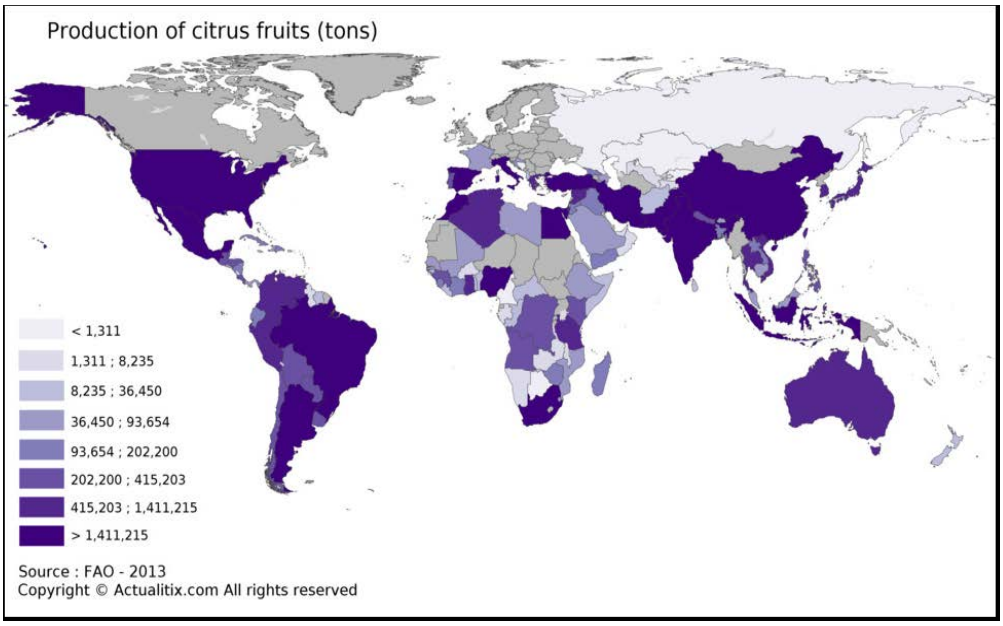


**Fig. 1.** World production of citrus fruits in tons (© Actualitix.com CC BY-NC-SA 4.0).

As a source of vitamin C and numerous other vitamins and minerals, citrus is marketed throughout the world as a beneficial healthy fruit. Because of their nutritional and organoleptic qualities, citrus fruits contribute to nutritional balance for both Northern and Southern populations. In Africa, citrus fruits are an important source of income to farmers with scarce resources. There are two clearly differentiated markets in the citrus sector: the fresh citrus fruits market (largely oranges) and the processed citrus products market, orange juice being the most widely consumed fruit juice in the world. Over the last two decades, small, easy peel and seedless citrus fruits (tangerines, clementines, mandarins and satsumas) have seen a consumer-driven rise in trade, at the expense of fresh oranges.

Consumption of citrus fruit juices has also increased, thanks to product convenience and healthiness, quality improvements, price competitiveness, promotional activity and technological advances in processing, storage and packaging. Among the major citrus varieties, only grapefruit has a level of processed utilization comparable to oranges (Lacirignola and D'Onghia, 2009).

## 1.2 History of citrus production and trends in East Africa

Citrus fruits, grown by commercial and smallholder farmers worldwide, are one of the most economically important fruit crops, creating employment in rural areas, income for resource-poor farmers and providing human nourishment (Kilalo *et al.*, 2009; Yesuf, 2013). Nigeria and South Africa are the largest citrus producers in Africa (Oke *et al*., 2020) and in East Africa, Tanzania has become the largest citrus producer after Kenya's higher elevation growers faced severe impacts from greening disease (Makorere, 2014). Citrus is grown within a broad band of approximately ±40◦ latitude of the equator in tropical and subtropical climates with ideal temperatures ranging from 20 to 34 degrees Celsius (Ackerman, 1938). Citrus is an important crop throughout East Africa, where production is mainly by smallholders, however yield is in decline due to pests and diseases (Ouma, 2008; Otieno, 2015). The yields of smallholder farmers in Africa often do not exceed 4–10 t/ha, while the crop has the potential of producing up to 50–70 t/ha in suitable countries practicing integrated pest management (IPM) programmes (Kilalo *et al.*, 2009).

Information (where available) is provided in Table 1 on the average area harvested, yield and production of citrus in countries typically included within the definition of East Africa.

**Table 1.** Average area harvested, yield and production of citrus in East Africa (average 2016–2018).

| Country | Area harvested (ha) (average 2016–2018) | Yield (hg/ha) (average 2016–2018) | Production (tonnes) (average 2016–2018) |
|---|---|---|---|
| United Republic of Tanzania | 50,190 | 206,387 | 443,219 |
| Kenya | 23,475 | 483,652 | 195,343 |
| Ethiopia | 77,72 | 247,911 | 42,699 |
| Somalia | 3,858 | 184,024 | 24,020 |
| South Sudan | 1,969 | 980,91 | 19,316 |
| Rwanda | 3,130 | 63,983 | 9,598 |
| Djibouti | no data | no data | 2,624 |

FAOSTAT (2019). Note: Data not available for Eritrea, Uganda or Burundi.

**Kenya**

Despite the decline in citrus fruit yields since the 1970s in Kenya, this remains a very important domestic commodity, ranked as the most extensively cultivated fruit crop in the country (Ajene *et al*., 2020a). The majority of the fruits produced in Kenya are consumed locally as a fresh product, and a smaller quantity is processed into juices, jam and marmalades (MMA, 2008).

The most common species of citrus planted are sweet oranges, lemons, limes, tangerines, grapefruits and pummelos. In 2014, total citrus production was estimated at 140,292 mt, valued at US$ 31.1 million (HCDA, 2014). These levels of production are insufficient to meet domestic demand and the country has moved from self-sufficiency to relying on imports of up to 44,000 tonnes (from Egypt, South Africa and Tanzania) to satisfy increasing popularity (Ouma, 2008; Makorere, 2014; Gitahi, 2016; TradeMap, 2019).

Low citrus yields and stagnation in production have been attributed to a range of factors including severe shortages of land, pest and diseases, declining government support, increasing competition from imports and lack of credit/investment capital (Ouma, 2008). Pests and disease infestation have resulted in reduced quality and quantity of outputs, as such the country's horticultural industry has encountered huge losses on domestic trade, and on potential export earnings whilst undermining the domestic systems competitiveness (Tschirley *et al*., 2004; Gitahi *et al.*, 2016). The highest citrus production is in the coastal, eastern and Rift Valley regions, although most smallholder farmers in North Rift abandoned citrus orchards due to low yields caused by diseases and insect pests (Mulaa *et al*., 2009). As far as fruit production is concerned, smallholder horticulture accounts for 40% of its production, with the market share of fruits accounting for 99% of the domestic market (Ouma, 2008) and prices are consequently determined by demand and supply forces. The production of citrus fruits contributes a modest 13% of the total fruit production area and has been suffering from low yields, with citrus greening being a prime constraint. In Tanzania by contrast, citrus fruits are widely grown over some 41,642.91 hectares of land to produce around 100.18 tonnes per hectare per year (Makorere, 2014). One study in the Kenyan counties of Makueni and Machakos highlight that for both small and medium scale farmers, citrus production is a major source of income - the majority of growers interviewed (89%) producing oranges on their farms with clementine the next most popular citrus fruit (23%), followed by lemon (13%), while a few produced tangerines (4%), grapefruit (1%), lime (%) and peach (1%). Similar trends were observed in both the surveyed counties, with a smaller percentage of orange producers in Makueni (Gitahi *et al*., 2019).

Losses due to pests and diseases are compounded by the use of non-budding planting materials, low fertilizer use and lack of irrigation, inadequate use of chemical pesticides and planting of unimproved cultivars of scions and root stocks (Tschirley *et al*., 2004; Ouma, 2008; Kongai *et al.*, 2018). This is exacerbated further by the use of infected planting materials, whose casual and uncertified distribution is recognized as a key transmission route for bacteria in particular. African citrus greening disease alone has significantly constrained Kenya's citrus fruit prospects. The disease was first observed in South Africa in the late 1920s (Oberholzer *et al.*, 1963) subsequently spreading to a number of other African countries (Bové, 2014a). Furthermore, in East Africa much citrus production is on smallholder farms which are characterized by intensive cultivation on available land close to other farms which is likely to contribute to the spread of both the vector and disease, especially when there is a lack of a coordinated management response (Ouma, 2008; Parsa *et al*., 2014).

**Tanzania**

Oranges were first introduced to Tanzania (Muheza District) in the early 1900s and by the late 1970s were of major economic importance (Ebony Consulting International, 2003). Today citrus fruits are one of Tanzania's most important horticultural cash crops, exceeding that of its neighbours Kenya and Uganda. It is grown in most regions in Tanzania with highest production in the Tanga region which is well-known for the variety of its citrus fruits, followed by the coastal region and Morogoro (22.41, 18.31, 10.9 t/ha citrus productivity, respectively) (Nyambo and Verschoor, 2005; Makorere, 2014). Out of an average 160,000 tons of citrus fruits produced per year in Tanzania, more than 85,000 tons are sold in the Kenyan markets (Makorere, 2014). Tanzania exports primarily oranges (mostly Washington Navel) to Kenya (accounting for 44% of imported oranges) with very limited export to other East African countries (and negligible exports outside of Africa) for local consumption but also for re-export to other regional markets (MMA, 2008). Indeed, exports from Tanga region in Tanzania to Kenya are estimated to account for 60% of oranges produced (MMA, 2008). Tanzanian oranges are delivered to Nairobi, Mombasa and Nakuru; during the peak season on a daily basis an average of 421,200,000 oranges (421,000 bags) arrive in Kenya (MMA, 2008). Tanzania also imports citrus from other countries such as South Africa. The Tanzanian domestic market includes primarily rural and urban consumption through informal markets, formal retail channels and institutional consumption, and the market of processed products which is not yet well developed (MMA, 2008). However, the citrus industry in Tanzania is not well developed, constrained by lack of irrigation and farmers' limited skills (Makorere, 2014). The most common varieties of oranges produced include Msasa, Nairobi, Valencia, as well as Pamba, Jaffa, Washington and Zanzibar. Yield is approximately 8.5 t/ha (MMA, 2008).

**Ethiopia**

In Ethiopia, citrus is one of the most economically-important fruit crops grown by smallholder and commercial farmers and includes sweet orange (82% of total citrus grown), mandarin, lime, lemon, grapefruit, citrus hybrids, sour orange and citron (Dagnew *et al.*, 2014). The main varieties of sweet orange grown include Valencia (61.1% of locations considered), an unknown variety (58.3%), Washington Navel (47.2%), Hamlin (44.4%), Pineapple (38.9%) and Jaffa (16.7%), with the most commonly-used rootstocks being Sour orange, Volkameriana and Troyer Citrange (Dagnew *et al.*, 2014). Approximately 70% of the industry is represented by the major citrus production areas of Afar, Somali, Oromia and Amhara Regional States (Bekele, 2007). Citrus farming occurs as part of mixed agriculture with other crops, vegetables and non-citrus trees. The majority of fruit produced (98%) is supplied to local markets such as Addis Ababa, Nazareth, Methahara, Diredawa and Harar, with fruits from the north central farms marketed at Dessie, Woldva and Mekele towns, with only a very small proportion (2%) from central east areas exported to neighbouring countries i.e. Djibouti and Somalia (Bekele, 2007). The industry is threatened by various diseases (Dagnew *et al.*, 2014).

**Somalia**

Grapefruit is the major citrus variety grown in the Mogadishu region of Somalia. Plantations were established in the 1970s with cultivars consisting of both old and new varieties such as Marsh seedless, Ruby and Redblush with sour orange and rough lemon rootstocks. In addition to grapefruit, small-fruited acid lime is the second most important citrus variety grown.

**Eritrea**

Citrus is one of the leading agricultural products in Eritrea (Lansford, 2017). Within the country the western escarpment and south-western lowland zone are particularly suitable for citrus production under irrigation by smallholders and medium scale irrigated plantations for internal market and export. Citrus is also produced in the north-western lowland zone under a hot arid climate and the greenbelt zone on the eastern escarpment of the central highland zone in the lower areas where the climate is suitable (Ministry of Agriculture, 1995). Citrus production activities are reported to have begun seriously from 1952 and by 1958 the De Nadai citrus farm in Elaberd was exporting over 3,500 tons to the Arabian Peninsula and Europe. By the late 1960s it is reported that the 1200-hectare plantations of De Nadai were the most productive in Eritrea (Negash, 1997). The Ministry of Agriculture reported the total cultivated land in Eritrea to be 410 ha (373 ha orange, 33 ha lemon, and 4 ha mandarin) and production 4,450 tonnes in 2003 with the average yield per ha for all citrus approximately 10.0 tonnes per ha and as such much below the international averages (Naqvi *et al.*, 2016).

**Uganda**

In Uganda, commercial citrus production (common sweet oranges, lemons and tangerines) began in the 1960s with over a decade of successful production followed by declining productivity and an eventual re-establishment of the industry in the 1990s (Njoroge *et al*., 2009; Kongai *et al*., 2018). Common varieties include Sweet Valencia, Washington Navel, Hamlin and the local orange (Kongai *et al*., 2018). Kongai *et al.* (2018) report that in eastern Uganda citrus farming is a smallholder enterprise whereby farmers produce citrus using traditional technologies with limited chemicals and irrigation and use of informally-distributed plant materials. Fruits are commonly sold fresh as value addition by processing is not established with a lack of coordination in the value chain. However, citrus is a profitable venture that can lead to improved farm income for smallholders as well as local employment opportunities (Kongai *et al.*, 2018). The total area under citrus fruit cultivation is approximately 10,000 ha with a total annual production of 10,000 tonnes (Njoroge *et al*., 2009).

**Rwanda**

In Rwanda the production of common sweet oranges mainly occurs in the south-west Cyangugu province with fruits consumed fresh locally and some processing into juice taking place (Njoroge *et al.*, 2009). Citrus production in Djibouti has grown at an average annual rate of 2.41% from 1,704 tonnes in 1999 to 2,676 tonnes in 2018 (Knoema, 2020a).

**South Sudan**

Depending on the agro-ecological zone, a wide range of food and cash crops are grown in South Sudan, including citrus. Here, citrus production has increased at an average annual rate of 7.08% from 13,000 tonnes in 2012 to 19,480 tonnes in 2018 (Knoema, 2020b). However, most activity in the rural sector is low-output subsistence agriculture. There is a lack of information on citrus production and it is therefore assumed that the citrus grown here would be similar to that of Sudan where the most important citrus fruit trees include sweet orange, grapefruit and lime with other citrus grown but on a much smaller scale including mandarin, lemon and pummelo (NBSAP, 2000).

**Burundi**

Limited information is available on citrus production in Burundi, however the Poverty Reduction Strategy Paper states that in order to improve economic growth, promotion of agricultural inputs was to be intensified which would include the introduction of rootstocks of citrus trees (IMF, 2009).

## 1.3 Understanding African and Asian citrus greening and their vectors

Citrus greening has been known, from its first description in the early 1900s in China, as huanglongbing (HLB, translated as “yellow shoot disease”), referring to the characteristic yellow colour of the new flush of growth on infected trees. The same disease is called mottle leaf in the Philippines, dieback in India, phloem necrosis and vein phloem degeneration in Indonesia, and citrus destruction in Thailand.

Practically all commercial citrus species and cultivars are sensitive, regardless of rootstocks; with grapefruit, sweet oranges, some tangelos and mandarins being the most susceptible, and limes, lemons, sour oranges and trifoliate oranges the least (Abdullah *et al*., 2009). By the 1970s, HLB had devastated many citrus-growing regions in Asia and Africa (National Academies of Sciences, Engineering, and Medicine, 2018) but for twenty years, during the 1970s and 1980s, the only way to confirm infection by HLB in trees showing symptoms was by Transmission Electron Microscopy (TEM) (Garnier and Bové, 1996). Characterization of the causal organism as a bacterium was eventually achieved by cytology coupled with electron microscopy (Garnier *et al.*, 1984a,b). Specific detection and identification of the African and the Asian HLB bacteria as Gram-negative organisms, belonging to a new genus of alpha division of Proteobacteria: "Liberibacter", or more precisely, "*Candidatus* Liberibacter" was subsequently made on the basis of their 16SrDNA sequences (Villechanoux *et al*., 1992). The term *Candidatus* indicated that the taxonomical identification of the bacterium could not be carried out with cultured organisms but involved molecular characterization. Within the "*Candidatus* Liberibacter" genus, the African bacterium and the bacterium from the Asian diseases represented two distinct species, which were named *Candidatus* Liberibacter africanus (*C*Laf) and *Candidatus* Liberibacter asiaticus (*C*Las) respectively (Jagoueix *et al.,* 1994, 1997; Gottwald, 2010). The subsequent evolution of PCR techniques allowed *C*Laf and/or *C*Las to be detected and identified appropriately (Jagoueix *et al*., 1996; Hocquellet *et al*., 1999). The disease spread to Oceania, South and North America and the name huanglongbing was officially adopted for all forms of the disease in 1995 (Bové, 2014b) following research demonstrating its infectious nature and transmissibility by graft inoculation (Lin, 1956), and by two citrus psyllids.

A third Liberibacter, *Candidatus* Liberibacter americanus (*C*Lam), was discovered in Brazil in 2004. The nomenclature adopted for the naming was on the basis of the continent from where each Liberibacter species had evolved. Later in 2005, the Asian HLB was reported for the first time in the Americas, namely in Florida and Sao Paulo, and by 2013, the disease was widespread in North, Central and South America. Subsequently, HLB is now widely used to describe the African, American and Asian forms of the disease wherever they occur, and is provided together with the specific causative agent to differentiate the strains (Fig. 2).

*C*Laf, which causes African citrus greening disease (AfCGD) is widespread in Africa. *C*Laf is historically known to be the most prevalent Liberibacter species on citrus on the continent and was first observed in South African citrus orchards in the late 1920s (Oberholzer *et al*., 1963). The disease associated with *C*Laf in South Africa is locally known as African greening (Oberholzer *et al*., 1963). Since its initial discovery, *C*Laf has been associated with citrus in 15 other African countries and the Mascarene islands (Graça, 1991; Garnier and Bové, 1996; Bové, 2014a; Roberts *et al*., 2017). In some areas in Kenya, whole orchards were lost due to HLB, while mild infestations caused up to 25% yield loss (Kilalo *et al*., 2009). Following the discovery of HLB in Kenya at Thika, Kamiti and Kitale in the 1970s (Schwartz, 1975), application of systemic and contact insecticides was recommended combined with removal of HLB-infected trees, planting disease-free nursery stock and abandonment of citrus production in areas above 800 m.a.s.l. that were known to be prone to colonization by the psyllid vector (Beige *et al*., 1984). Within the East African region, HLB was first reported in Tanzania (Schimwela, 2016); Rwanda (Aubert, 1987), Burundi (Bové, 2006) and Angola (Fourie *et al*., 2020), with *C*Laf as causal organism and *T. erytreae* as vector.

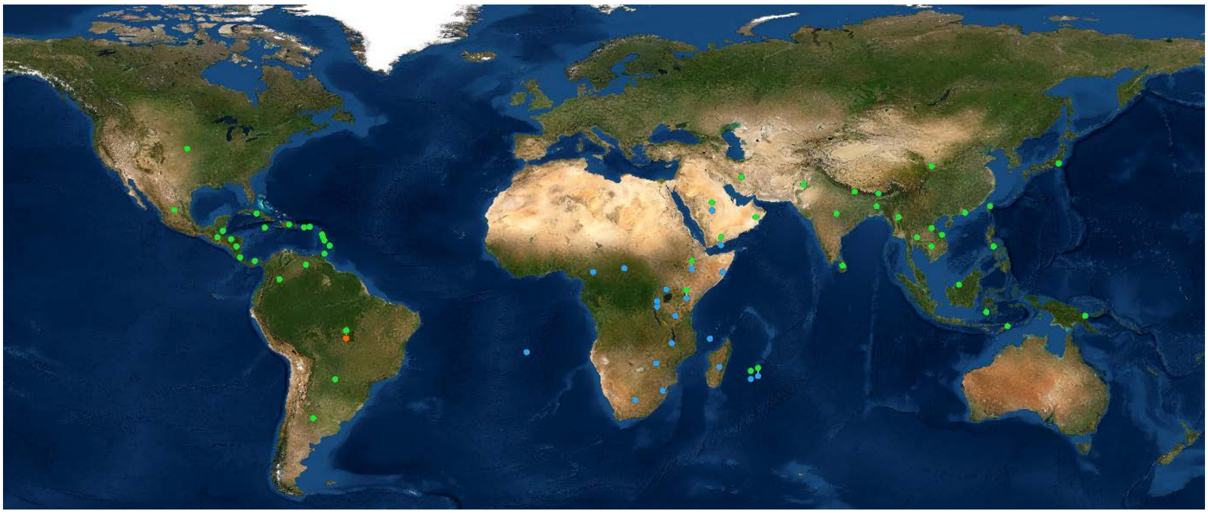

**Fig. 2.** Distribution of *Candidatus* Liberibacter asiaticus [green], *Candidatus* Liberibacter africanus [blue] and *Candidatus* Liberibacter americanus [orange] (CABI ISC data, August 2020).

A number of new lineages of the *Candidatus* species have also been reported including five subspecies of *C*Laf identified from indigenous rutaceous trees in South Africa i.e. *C*L africanus subsp. capensis (*C*LafC) (Garnier *et al*., 2000), *C*L africanus subsp. clausenae (*C*LafCl), *C*L africanus subsp. vepridis (*C*LafV), *C*L africanus subsp. zanthoxyli (*C*LafZ) (Roberts *et al*., 2015) and *C*L tecleae (*C*LafT) (Roberts and Pietersen, 2016). Currently, little is known about these '*C*L africanus' subspecies regarding their host range, transmission and disease-causing potential to citrus, although *C*LafCl has been reported from citrus in Ethiopia, Kenya and Tanzania (Shimwela *et al.,* 2016; Roberts *et al.*, 2017; Ajene *et al.*, 2020b).

Although the liberibacters remained native to the particular zones where they were discovered, in modern times they have spread, along with their psyllid vectors, to new areas, along with their susceptible crops (aidoo). *C*Laf is transmitted by the African citrus triozid *T. erytreae* (del Guercio) (Triozidae), also known as the African citrus triozid (ACT) (Aidoo, 2020b). By contrast, *C*Las is transmitted by the Asian citrus psyllid (ACP) *D. citri* Kuwayama (Liviidae) (Graça & Korsten, 2004) (Figs. 3 and 4). However, both ACT and ACP have been shown to be capable of transmitting both forms of the disease experimentally (Lallemand *et al*., 1986).

ACP infests species in at least 10 genera in the family Rutaceae (which contains about 160 genera including Citrus) with variable suitability for oviposition, development and reproduction. This insect requires 2 to 7 weeks to develop from egg to adult, adults live several months, and there are 9 or 10 generations per year. Adults fly 25 to 50 m regularly, but have been found to fly up to 100 m towards new leaf flushes over a 3-day period (Boina *et al.*, 2009). In laboratory conditions, *D. citri* are capable of flying continuously for up to 2.4 km without wind assistance (Martini *et al*., 2014; Lewis–Rosenblum *et al.*, 2015) and further long-range dispersal of psyllids might be facilitated by wind (Gottwald *et al.*, 2007). It is interesting to note that psyllids have been collected in dogue nets towed behind light aircraft flying at a height of 300m (White, 1970). ACT can disperse up to 1.52 km within 7 days in the absence of host plants (Berg and Deacon, 1988). Trapping data from Florida indicate no consistent ACP seasonal movement patterns, which means continuous monitoring is required. Multimodal sensory inputs contribute to host finding; ACP orient to leaf flush via volatile signals and assess plant suitability using gustatory and visual cues. Ovipositing females prefer the plant species on which they developed, but preferences can shift after exposure to an alternative host.

Adults are attracted to colours within the reflectance spectra of rutaceous plants. This has formed the basis for monitoring of ACP and ACT with traps. A survey in Kenya compared eight differently coloured double-sided sticky traps to evaluate which colour was most effective for detecting ACT and ACP, particularly at low densities. Yellow and green traps captured more ACTs and ACPs than any other trap type (Aidoo *et al.*, 2020b). Infected plants are more attractive to ACP due to HLB-induced volatiles. However, infected trees are less suitable for ACP development, and psyllids leave infected plants shortly after pathogen acquisition, promoting pathogen spread.

*T. erytreae* is the only *Trioza* species to feed and develop on Rutaceae plants, not only citrus, but also citrus relatives, including *Vepris lanceolata* (Lam.) {= *Vepris undulata* (Thumb.), = *Toddalia lanceolata* (Lam.)} and *Clausena anisata* (Willd.), two preferred, native hosts of the African citrus psyllid. *Fagara capensis* (Thumb.) {= *Zanthoxylum capense* (Thumb.)} is also an adequate indigenous host for *T. erytreae* development (Moran, 1968). In Kenya, a recent study also found this insect feeding on *Ficus thonningii* (Blume), *Ficus sycomorus* L. (both Moraceae), *Stephania abyssinica* (Quart, Dill & A. Rich) Walp. (Menispermaceae), *Murraya koenigii* (L.), *Teclea nobilis* (Del.), *Calodendrum capense* (L.f.) Thunb. and *Vepris bilocularis* (Wright & Arn.) Engl. (Rutaceae) (Aidoo *et al.*, 2019). Thus, before citrus and its hybrids (which are not indigenous to Africa) were introduced into Africa, *T. erytreae* had at least two native rutaceous hosts to complete its development: *V. lanceolata* and *C. anisata*. These two plants are among the original host plants of *T. erytreae* (Moran, 1968). *V. lanceolata* is not only a preferred host of ACT, it has also been reported as a host to the HLB bacterium, *C*Laf (Korsten *et al*., 1996). *T erytreae* also develops well on *Citrus limon* and must have been well pre-adapted to this host at the time of the introduction of citrus to Africa. For this pre-adaptation purpose, *F. capensis* was almost as suitable as *C. limon* for the development of *T. erytreae* (Moran, 1968).

The African continent remained free from *C*Las until 2010, when detection of this bacterium was reported from citrus trees in northern Ethiopia (Saponari *et al.*, 2010). *C*Las has recently been reported from Kenya (Ajene *et al.*, 2020a), while the HLB vector *D. citri* is reported from Ethiopa, Tanzania, Kenya and Nigeria (Rwomushana *et al.*, 2017; Ajene *et al.,* 2020c; Oke *et al.*, 2020). Secondary transmission is also possible by grafting and colonization by parasitic dodder plants (*Cuscuta* spp.) (Zhou *et al*., 2007; Graça, 2008).

## 1.4 Differentiating symptoms of African and Asian greening in East Africa

The African form of the disease (*C*Laf) and its associated psyllid vector, ACT, are both heat-sensitive, occurring in regions of cool, moist climate, often on highlands, with temperatures below ~30°C (Bové, 2014a). The Asian form of HLB (*C*Las) and its Asian psyllid vector, ACP are both heat-tolerant, occurring at temperatures above 27°C (Batool *et al.,* 2007), although they are more sensitive to high rainfall and humidity (Moran and Blowers, 1967; Catling, 1972; Aubert, 1987; Graça, 1991).

Symptoms of HLB are influenced by temperatures at which affected trees grow. The two *Candidatus* species, *C*Las and *C*Laf both cause the characteristic symptoms of chlorosis that resemble zinc deficiency and mottling on leaves, as well as the poorly developed root systems and decay from the rootlets (Graça, 1991; Batool *et al.*, 2007). The blotchy mottle pattern on one side of the leaf midrib is not symmetrical to that on the other side and this asymmetry distinguishes blotchy mottle from nutrient deficiency. In early stages of infection, blotchy mottle might be the only leaf pattern to be seen. Twig dieback, fruit drop, irregularly-shaped (lopsided) fruit, reduced fruit size and lower internal fruit quality with bitter taste are other typical symptoms (Fig. 6). However, the Asian form of greening causes more extensive dieback and tree death. In the case of African greening, fruits remain immature and green, and seeds are aborted and stained (Batool *et al.*, 2007). Usually the actual causative agent will be identified using molecular tools. Typically, the nymphs of *T. erytreae* live in individual depressions on the undersides of host plants (Fig. 5), whereas nymphs of *D. citri* tend to colonize the stems of new growth and never produce individual pits on the leaves (Halbert and Manjunath, 2004).



**Fig. 3**. Adults and nymphs of *D. citri* on citrus plant (credit: I. Rwomushana, CABI); **Fig. 4**. Adult *D. citri* on citrus flush (credit: D. Hall, USDA-Agricultural Research Service).


**Fig. 5.** Nymphs of *Trioza erytreae* (African citrus triozid) on leaf (credit: Peter Stephen, CRI).

## 1.5 Huanglongbing symptoms and crop damage

Greening-affected trees generally exhibit open growth, stunting, twig dieback, sparse yellow foliage or severe fruit drop (Batool *et al.,* 2007). After inoculation of the phloem sieve tubes in the citrus host during feeding of the psyllid vector, *Ca.* Liberibacters exploit phloem cellular processes for nutrient acquisition. *C*Las levels increase rapidly, however infected trees remain asymptomatic through the early disease phase. Indeed, these multiple asymptomatic infections, incomplete systemic distribution within trees and prolonged incubation period can make detection of HLB difficult. Sometimes a citrus plant will express with typical HLB infection such as yellow shoots and blotchy, mottled, narrow leaves, sometimes with vein yellowing, on one branch while other parts of the tree remain symptomless. As the bacterial colonization progresses systemically, the canopy also becomes thin and chlorotic, tree growth slows, leaves remain reduced in size and leaf tips become necrotic (Halbert and Manjunath, 2004; Bové, 2006; Gottwald *et al*., 2007). Mathematical models based on HLB epidemiology have shown that once detectable infections occur in 5% of trees within an orchard, it is likely that 90% of the orchard is infected (Craig *et al*., 2018), even in the presence of control.


**Fig. 6.** Fruit of HLB-infected citrus tree showing colour inversion (credit: USDA-Animal and Plant Health Inspection Service (APHIS)).

In the early stages of the disease, a clear diagnosis may be difficult to achieve because of mildness of symptoms and resemblance to other conditions. McCollum and Baldwin (2017) found that HLB symptoms were more pronounced during cooler seasons than in warmer months. Leaves can thicken and veins enlarge and appear corky. Later, yellow blotches appear between veins that remain green, similar to zinc deficiency or other nutritional deficiencies and leaves may drop as twig ends become necrotic (Gottwald *et al*., 2007). The root systems are poorly developed, showing very few fibrous roots, likely due to nutrient starvation (Graça, 1991; Batool *et al*., 2007). In some cases, green colour develops on fruit at the peduncular rather than the stylar end, causing inverted colouration. Fruit from diseased trees are marked by their bitter taste, they are reduced in size, may be asymmetrical and contain small, brownish/black aborted seeds. As the disease progresses, fruit yield and quality decline (Timmer *et al*., 2000; Halbert and Manjunath, 2004; Bové, 2006).

*Ca.* Liberibacter spp. multiply in the sieve tubes of infected plant hosts, squeezing from sieve cell to cell through sieve pores. As a host response to the spread of the bacterium in the vascular system, an increase in callose deposition occurs and as the bacteria multiply, the flow of phloem sap between cells is impeded, disrupting the movement of sugars (Koh *et al.*, 2012). *C*Las also suppresses the immune response of the tree allowing for a long incubation period. One of the predominant biochemical responses to HLB is the excessive accumulation of starch in the aerial plant parts, due to the upregulation of glucose-phosphate transport, which is involved with the increased entrance of glucose into this pathway (Martinelli and Dandekar, 2017). It is this imbalance in sugar transport and metabolism and persistence of starch in leaves that results in root starvation, severe health decline and eventual death (Etxeberria *et al*., 2009; Fan *et al.,* 2010; Zheng *et al*., 2018). Changes in the secondary metabolite profiles of citrus and suppression of defence responses have also been found in *C*Las-affected citrus (Nwugo *et al*., 2013).

*C*Las must first be ingested and then translocated into the insect's gut tissues. Subsequently the bacteria can replicate and systemically infect the insect, moving to the salivary tissues from which they can infect other host plants (Ammar *et al.,* 2011). Most vectored plant pathogens can be acquired by their vectors at or just prior to the onset of symptoms. Until then, the plant might be infected, but it is not spread to other plants. The time between infection and the time when vectors can acquire the pathogens is the latent period, whilst the incubation period refers to the time between infection and appearance of symptoms (National Academies of Sciences, Engineering, and Medicine, 2018). With HLB, the latent period is equal to one generation of psyllid vectors (Lee *et al*., 2015), and the incubation period is long and variable, up to at least 6 years (Shen *et al*., 2013). Lee *et al*. (2015) reported on the phenomenon of infective colonization events - in other words, HLB is passed on from adults to their progeny as the nymphs acquire *C*Las from their feeding site, previously infected by their parents (Lee *et al*., 2015).

The two main factors regulating psyllid populations are availability of young growing shoots for oviposition and temperature and depending on these, the latent period can occur in 2–3 weeks. A single female psyllid is capable of producing 800 to 1,000 eggs over her lifespan (Tsai and Liu, 2000). The increase in numbers of infected psyllids can be enormous, especially if abundant new colonization sites are available for emerging infected adults. Late nymphs and adults have a propensity to acquire *C*Las on young flush tissue and are attracted to the yellow-green colour of HLB-infected trees (Graça, 1991), which explains their attraction to yellow/green sticky traps (Sétamou *et al.*, 2016). *C*Las infection of the citrus tree further induces the release of methyl salicylate, a plant host volatile, which renders infected plants more attractive than uninfected plants. However, infected trees are less suitable than uninfected plants as hosts for development of ACP, and the tendency of psyllids to leave infected plants shortly after pathogen acquisition may promote the spread of the pathogen (National Academies of Sciences, Engineering, and Medicine, 2018). As acquisition time increases, so does the percentage of psyllids becoming infected with *C*Las (Brlansky and Rogers, 2007). Additionally, adult psyllids have been shown to be capable of acquiring *C*Las from citrus that is infected with the pathogen yet not showing symptoms but due to the uneven distribution of the pathogen within a plant, not all parts of the tree will serve as an inoculum source at any given time (Brlansky and Rogers, 2007). The disease tolerance of different citrus varieties varies significantly and some have been found to accumulate a greatly reduced pathogen load compared with susceptible varieties (Albrecht and Bowman, 2012) and show less severe symptoms (Fan *et al.,* 2012).

HLB impact is dependent on several other variables including age of tree at first infection, levels of tolerance and environmental factors. These variables may allow some trees to maintain the same or similar levels of productivity several years post-infection, while others may decline rapidly. HLB causes fruits to drop prematurely, resulting in a 30–100% yield reduction. HLB-infected trees become unproductive within 2 to 4 years after the onset of the disease and young infected trees typically do not reach productive age.

Tree mortality can occur several months to years after infection (McClean and Schwarz, 1970; Graça, 1991; Bové, 2006; Batool *et al.*, 2007; Bassanezi *et al.*, 2011; Liao and Burns, 2012). Huanglongbing can infect all commercial citrus cultivars and causes substantial economic losses by shortening the production life span of infected trees (Bové, 2006). Without any control, in younger orchards, tree death can result in 50% of plantings within 5 years, and may take longer in older groves (Gottwald, 2010). In China, without any control, it took only about 5 years for an orchard to reach 100% infection. In Reunion, the time needed to reach 100% infection was estimated to be 13 years while the average time worldwide is reported as 8 years (Bové, 2006).

## 1.6 Current spread and distribution of HLB in Africa

Saponari *et al*. (2010) reported the presence of *C*Las in samples from symptomatic sweet orange budwood trees and orchard plants collected in April 2009 from Tigray and North Wollo in northern Ethiopia. These were the first records of *C*Las for the country and for the African continent. The authors at the time recommended prompt eradication of symptomatic trees and further surveys to determine the full distribution of the disease and its vectors, however, Bové reported failure in encouragement of Ethiopian authorities to do this in 2012 and 2013. A survey in November 2017 of the African citrus greening vector *T. erytreae* was carried out in the Amhara Region (central to north-western Ethiopia) by Ajene *et al*. (2019). Screening of *T. erytreae* samples collected from sweet orange, lemon and tangerine trees revealed them to be carrying *C*Las, the first time this had been reported in field populations of the psyllid. Previous research has demonstrated the capacity of *T. erytreae* to transmit *C*Las (Massonie *et al*., 1976) and this development has brought additional concerns of *T. erytreae* as an alternate route of HLB proliferation in the absence of, or in addition to the primary *C*Las vector, *D. citri*. In 2020, following reports of presence of the primary HLB vector from Tanzania and Kenya, Ajene *et al*. (2020b) surveyed citrus-growing regions in Ethiopia for the psyllid and found it to be present in five locations in North and South Wollo in the north of the country, at altitudes between 1,619 and 2,112 m.a.s.l – higher than is typical for the psyllid in its native range. The source of *D. citri* was not clear, but the psyllid may have arrived on imported plant materials. Vector management was recommended, but the spread of *D. citri* southward is expected to be imminent (Ajene *et al*., 2020a). In addition, a recent study has shown that *C*Las is increasingly widespread in Ethiopia, present in regions of the country distant from previously surveyed areas and thriving at higher altitudes with cooler temperatures (Ajene *et al*., 2020b).

The first detection of *D. citri* in Eastern Africa was reported from 2014 and 2015 surveys in Tanzania by Shimwela *et al*. (2016) at altitudes of 300–600 m.a.s.l. around Morogoro in the east of the country. Rwomushana *et al*. (2017) conducted further surveys for the psyllid from 2015 to 2016 in Kenya, Tanzania and Zanzibar. *D. citri* was found to be present in Kenya at six sites: four in Kwale county in the south-east near to the coast and the Tanzania border; one in Nairobi county; and one in Kericho county in the west, at altitudes ranging from 20–1,666 m.a.s.l. In Tanzania, *D. citri* was most prevalent in the Morogoro region from which it was first reported but was also present to the east to Dar es Salaam and Zanzibar and south to Kibiti, at altitudes ranging from 19–668 m.a.s.l. It was apparent from this study that the distribution of *D. citri* in these countries was expanding (Rwomushana *et al*., 2017). Between 2016 and 2018, Rasowo *et al*. (2019) surveyed citrus in Kenya and Tanzania for greening symptoms and vectors and concurred that *D. citri* is spreading fast in the region and adapting to new geographic areas and ecologies. Of great significance to both Kenya and Tanzania in the immediate term is the recent detection of *C*Las at sites in the warm coastal region of Kenya, close to the border with Tanzania (Ajene *et al*., 2020a). With *D. citri* also present in this area, there is a high risk of HLB proliferation in the region.

Kalyebi *et al*. (2016) reported presence of *C*Las in *T. erytreae* specimens from Washington Navel and rough lemon at two sites in eastern Uganda - a first record of the disease in the country - however, subsequent research has suggested that this was a misidentification of *Candidatus* Liberibacter africanus subsp. clausenae (*C*LafCl) (Roberts *et al*., 2017; Ajene *et al*., 2020a) and to date it is not apparent that *C*Las or *D. citri* are present in the country.

The current distribution and ongoing spread of both *C*Las and its primary vector *D. citri* in multiple countries, compounded by *T. erytreae's* potential to vector HLB including at higher altitudes is cause for huge concern. Furthermore, the predicted suitability of many important citrus-growing regions in East Africa to the disease and its main vector (as described in Section 1.7), warrants urgent action to contain and limit the impacts of HLB in the region.

Of great significance to Nigeria – a major citrus producer – and its neighbours in West Africa is the recent arrival of *D. citri* (Oke *et al*., 2020). The psyllid was collected from sites in Oyo State in the south-west of the country in October 2019. The psyllids were assayed for presence of *C*Laf, *C*Lam and *C*Las, but fortunately none tested positive for any of these liberibacters. The tropical climate prevailing in citrus-producing areas of Nigeria and other West African countries may favour the rapid spread of this psyllid and are also expected to be ideal for HLB should the disease arrive in the region (Narouei-Khandan *et al*., 2016; Ajene *et al*., 2020d; Oke *et al*., 2020). For this reason, a rapid response to control *D. citri* and the development of rigorous plans to manage HLB in the region, should it arrive, would be valuable.

## 1.7 Modelling environmental suitability and potential distribution of HLB and its vector

A challenge to the African citrus industry is the current lack of knowledge on the habitat suitability for Liberibacter infection across the continent (Ajene *et al*., 2020d). In order to make effective risk assessments of potential bio-security threats, prediction models are extremely useful and help to inform management strategies to minimise future losses, in addition to estimating the potential impacts of climate change on species distributions (Graça and Korsten, 2004; Ajene *et al*., 2020d).

Although insect vector transmission is one of the major contributors to disease spread and previous studies have focused on this, it is also important to consider the environmental requirements of the pathogen (Gottwald, 2010). Here, consensus modelling for the vector and pathogen provides a more robust view of potential disease distribution (Ajene *et al*., 2020b). Narouei-Khandan *et al*. (2016) conducted the first study assessing the potential global risk of HLB (*C*Las) and ACP establishment in citrus-growing areas using two types of correlative modelling approaches to assess climate suitability: (i) Maximum Entrophy (MaxEnt) as a presence-only or a presence-background model (Phillips *et al*., 2006) and (ii) Multi-Model Framework (Worner *et al*., 2010) whereby from nine models the Support Vector Machine (SVM) achieved the highest performance rank.

Predictions for potential HLB (*C*Las) distribution for both models were that central and eastern parts of Africa would be environmentally suitable for the Asian form of HLB. Highly suitable areas predicted for ACP establishment were the lowlands in Central and Eastern Africa, in addition, large areas in Central and West Africa, and coastal areas in Northern Africa were predicted as climatically highly suitable for the psyllid. Thus, without preventative measures these predictions suggest *C*Las could establish widely in Africa with potential hotspots in Central and South-eastern Africa (Fig. 7) (Narouei-Khandan *et al*., 2016).

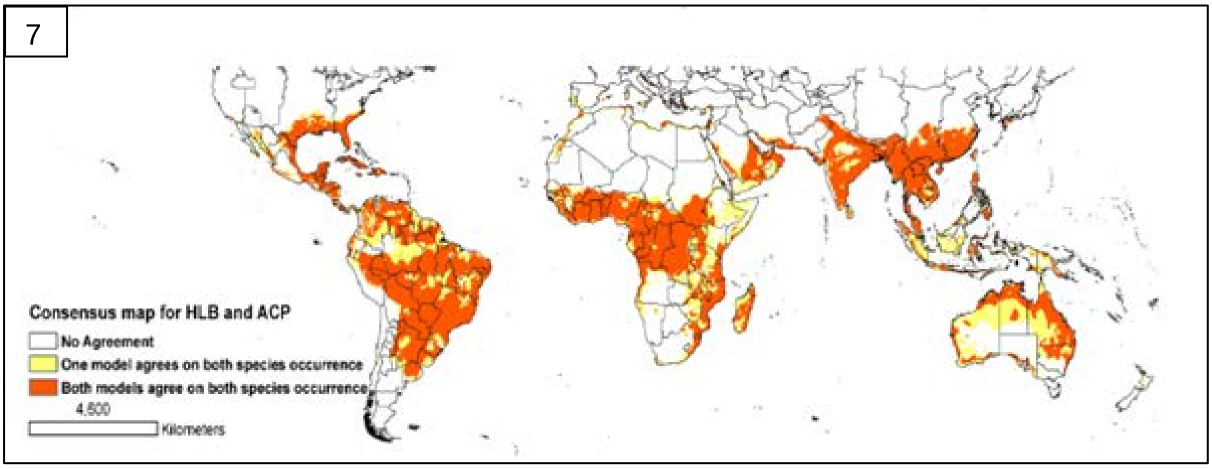


**Fig. 7.** Consensus model showing the hotspot areas where one or two models (MaxEnt and SVM) agree on the probability of both citrus huanglongbing (HLB) caused by *C*Las and the Asian citrus psyllid (ACP) occurrence (from Narouei-Khandan *et al.,* 2016).

MaxEnt and Multi-Model (SVM) predictions by Shimwela *et al*. (2016) predict suitability for *D. citri* establishment in the majority of citrus-growing areas in Africa. They conclude that establishment of *D. citri* and *C*Las is highly likely in East Africa, part of South-central Africa, and some areas of West Africa, where climatic conditions would be suitable (Shimwela *et al*., 2016). Ajene *et al*. (2020a) using a three-model consensus (MaxEnt, BIOCLIM and Boosted Regression Trees) again predict potential distribution of *C*Las in large areas of Central, Eastern and Southern Africa and some parts of Western Africa where marginal to optimal habitat suitability is demonstrated for HLB establishment (Fig. 8). Predictions under climate change scenarios indicate in a moderate scenario, hotspots were predominantly in Southern Africa, whereas in the extreme scenario, hotspots were predominantly in Western, Central and Eastern Africa.

A further consensus model considering the environmental requirements for both the vector and pathogen indicate large areas of optimum habitat suitability for the pathogen in coastal and western Kenya where citrus production is high with increased risk of spread into central Kenya.

Large areas of optimum habitat in citrus-producing areas were also identified in Ethiopia including Nura-Hera, Awasa, Jimma and Dire Dawa. In Tanzania large areas were found to be marginal for the optimal suitability for the pathogen, but the island of Zanzibar showed high suitability. In eastern Uganda, for the major citrus-producing region of the country, the model showed marginal suitability for *C*Las (Fig. 9) (Ajene *et al*., 2020b).

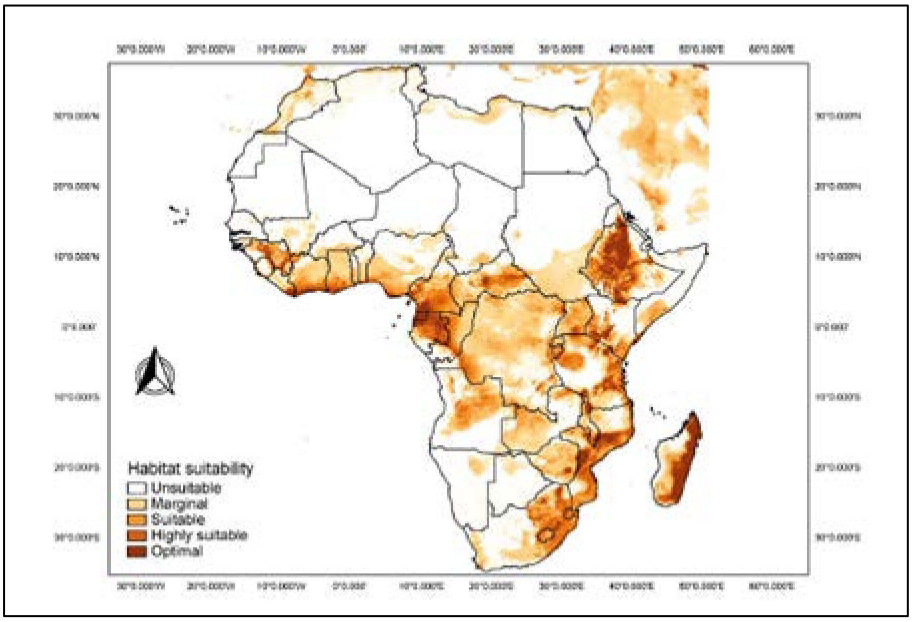


**Fig. 8**. Potential distribution of Huanglongbing in Africa, as predicted by three-model consensus (BIOCLIM, MaxEnt and Boosted Regression Trees) (from Ajene *et al*., 2020a).

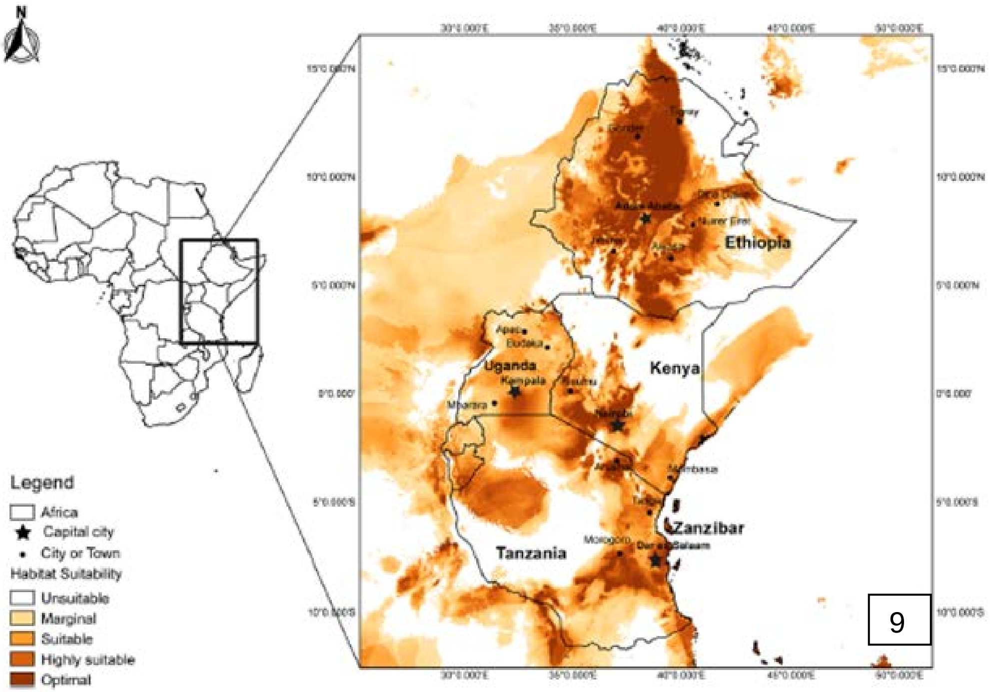


**Fig. 9.** Potential distribution of Huanglongbing associated with *Candidatus* Liberibacter asiaticus in Eastern Africa, as predicted from the current occurrence locations using global 50-year climate data with MaxEnt (from Ajene *et al*., 2020b).

Thermal niche maps produced by Taylor *et al*. (2019) support the model findings. Here, climate-predictive mapping (where transmission can occur between 16°C and 30–33°C with peak transmission at approximately 25°C) highlights highly suitable areas for HLB establishment including large citrus-producing regions near the equator in many African countries that are highlighted as not only permissive, but highly suitable for HLB for 12 months of the year (Figs. 10 and 11).

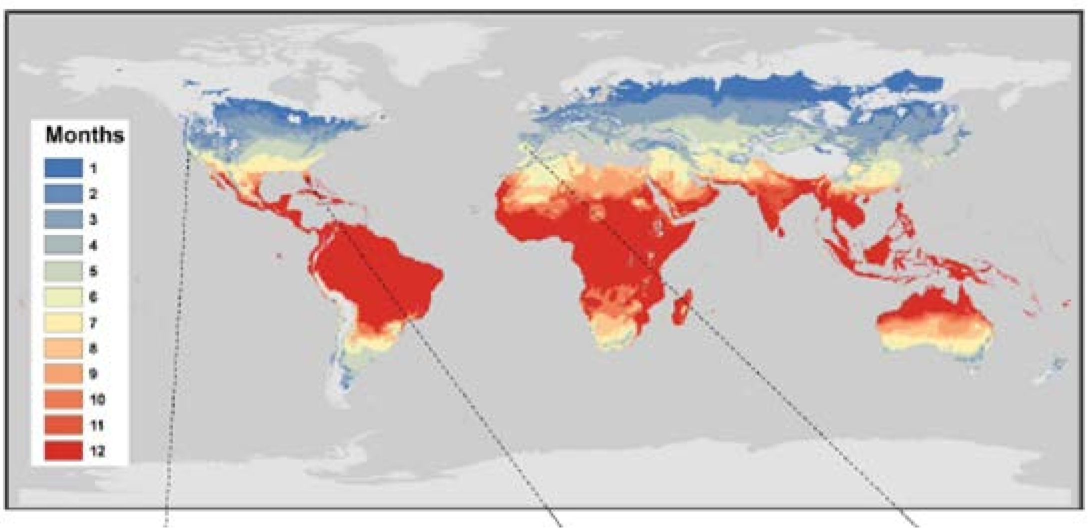


**Fig. 10.** The number of months per year that locations have permissive temperatures according to Taylor *et al*.'s H11 S(T) model (from Taylor *et al*., 2019).

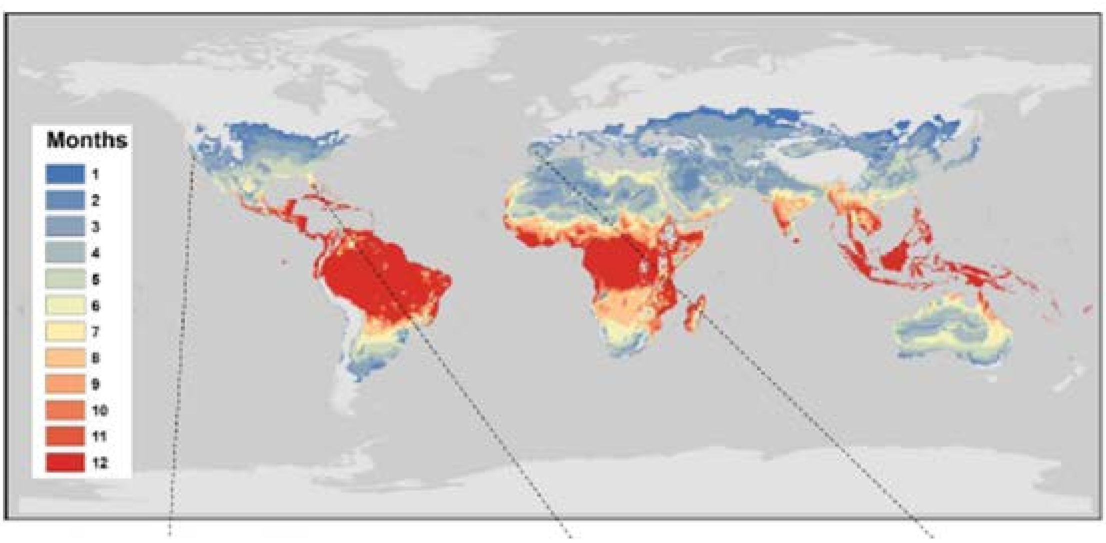


**Fig. 11.** The number of months per year that locations have highly suitable temperatures according to Taylor *et al*.'s H11 S(T) model (from Taylor *et al*., 2019).

In summary, environmental modelling allows the prediction of the potential distribution of HLB associated with *Candidatus* Liberibacter asiaticus. The findings from the studies presented here suggest that both the psyllid vector and pathogen have high probability of wide establishment in important citrus-producing areas of Africa and that their distribution would be further facilitated by changing climatic conditions.

# 2 Impacts of HLB on citrus and other socio-economic variables

## 2.1 Documented impacts of Huanglongbing in other regions

Asian citrus psyllid poses a major threat to citrus everywhere and is the prime focus of citrus pest management wherever it is present, with a large portion of economic losses in citrus production on the Asian and American continents attributed to Huanglongbing (Bové, 2006).

Globally, citrus producers are facing serious problems with the emergence of the HLB disease (Teixeira *et al*., 2008; Bassanezi *et al*., 2011). The United States is a major global citrus producer with the 2018–2019 crop valued at \$3.35 billion. HLB was responsible for the decrease in the production of oranges for processing in the United States from around 8 to 2 billion tons from 2007–2008 to 2017–2018, a drop of more than 70% since HLB was detected in 2005 (Hodges and Spreen, 2015). The fresh fruit market also decreased by 21% during the same time interval. This market was less impacted than the rest of the citrus industry because, in the United States, around 90% of the oranges produced in Florida, the state with the largest prevalence of HLB, are processed for juice, while California supplies oranges for the fresh market (USDA-NASS, 2018). Detection of HLB in Florida in 2005 impacted the entire Florida citrus industry, with its citriculture practices changing overnight. The result was intensive pesticide applications, aggressive removal of citrus greening-positive trees, and the complete switch from outdoor to indoor citrus nursery operations (Hall *et al*., 2012). Singerman and Useche (2016) reported a threefold increase in the price of a box of oranges since HLB had been detected in the United States. Economic loss from systemic infection is generally considered as a function of disease incidence (Qureshi and Stansly, 2020). A vector such as *D. citri* is a pest in its own right and its damage potential is increased in proportion to the number capable of transmitting disease. For a perennial crop such as citrus, there is the additional challenge for vector management in that disease incidence is cumulative rather than reset every cycle, as in annual crops. A preventive approach is therefore more favourable for citrus, especially early on, as the biological and economic impact of disease is greatest on young plants.

Eradication of HLB-infected and nearby trees in Florida eliminated one-tenth of citrus production capacity by 2008 and in Florida alone, HLB has caused a cumulative loss of \$2.994 billion in grower revenues over the 2006–2007 to 2013–2014 period, an average of \$374 million per year (Hodges *et al*., 2014), whilst a 2016 economic impact analysis reported losses of more than \$4.6 billion and over 30,000 jobs. Growers in other orange-growing states such as California, Texas and Arizona are also becoming increasingly affected by HLB. In California, economic losses amounting to \$2.7 billion due to Asian greening have been reported (Lopez and Durborow, 2015). In the past decade in the US, HLB has caused around a 21% decrease in the fresh citrus fruit market and about a 72% decline in the production of oranges used for juice and other products (Dala-Paula *et al.,* 2019). Several unsuccessful attempts have been made to cure or reduce HLB symptoms in Florida, where most citrus growers have chosen to live with diseased trees rather than eliminate them and this has proved the Achilles heel in the fight against HLB.

In China, where the disease originated, top-producing Jiangxi Province had lost 25% of its groves as of the end of 2018, with the destruction of a hundred million commercially grown citrus trees reported in Asia (Zhang *et al*., 2010).

In 2004, the first discovery of the disease in the Western Hemisphere was in São Paulo, Brazil. Since then, the disease has eliminated 52.6 million sweet orange trees, a 31% reduction in area. The psyllid vector was reported in São Paulo and Florida as early as 1942 and 1998, respectively (Bové, 2006; Tansey *et al*., 2017) however. *C*Lam was the most prevalent bacteria species in Brazil in 2005, which initially affected more than 90% of the infected trees, decreasing to 60% in 2007. During this period, there was an increase in *C*Las infection, from 5 to 35% of the infected trees, while a combined infection remained practically the same at 5% (Coletta-Filho *et al*., 2010; Gasparoto *et al*., 2012). The detrimental effect of HLB on citrus was further evidenced by the loss of a million *C*Las-infected citrus trees in São Paulo within 3 years (Gottwald *et al*., 2007). The Brazilian industry responded rapidly however, with sensitive molecular tools for diagnosis (PCR) in place within months of the first reports and in less than a year the Brazilian Government enacted legislation to enforce eradication, mitigation and prevention measures. As a result, the incidence of greening within all the major citrus-growing estates has massively declined but the disease took its toll nonetheless and the number of citrus farms declined from 14.6 thousand in 2007 to 9.5 thousand in 2018 with the greatest impact on small and medium-sized citrus growers that were unable to control and cope with the damage caused by HLB (Bassanezzi *et al.,* 2020).

In South Africa, severe losses from African citrus greening were experienced (30–100% in some areas) in the 1930s, 40s and 50s (Graça, 1991). Subsequently, control strategies have helped maintain HLB incidence at economically-acceptable levels despite heavy losses up until the late 1970s (Schwarz, 1967; Buitendag and von Broembsen, 1993). This is in part due to the temperature sensitivity of *C*Laf (Schwarz and Green, 1972) and its natural vector, *T. erytreae*, limiting the occurrence of HLB to cooler production areas (McClean and Oberholzer, 1965), combined with the effective control of *T. erytreae*.

In Saudi Arabia, all sweet oranges and mandarin trees had been lost by 1986 leaving only limes (Aubert, 1994). In Indonesia alone, HLB has resulted in the destruction of 3 million trees (Tirtawadja, 1980).

In the Philippines, citrus culture has been drastically limited by HLB. Between 1961 and 1970, the area planted to citrus was reduced by over 60%, and in 1971 over one million trees were destroyed in one province alone due to HLB (Graça, 1991). Similarly, in certain areas of Indonesia, the incidence of the disease is high. For instance, Tirtawadja (1980) reports the destruction of 30 million trees (Graça and Korsten, 2004). In northern Bali, almost 100% of mandarin trees planted in 1990–1991 were severely affected in 1996. In India, the disease is widespread (Bové *et al*., 1993; Varma *et al*., 1993) with reported catastrophic losses (Graça, 1991). India revealed that 83–95% of the 25% yield losses in citrus were associated with *D. citri* through direct damage or sooty mould fungus (Khan *et al.,* 2014). In the Pokhara Valley of Nepal, a major mandarin-growing area, trees show HLB symptoms before they are 10 years old. The trees are replaced and the disease reoccurs on new trees. This cycle of replanting has occurred several times since the disease was introduced from India in the 1960s (Regmi *et al*., 1996). In Thailand, HLB has caused severe destruction of citrus since its arrival in the 1960s, with typical yields low and groves commonly entering decline and becoming unprofitable as early as 5–8 years after planting. The north of the country has been very badly affected and many citrus areas have gone out of production (Roistacher, 1996).

Losses due to HLB have been more extensively documented in Asia than Africa, however it is estimated that by the early 1990s more than 60 million trees had been destroyed by the disease globally (Graça and Korsten, 2004).

## 2.2 Documented impacts of Huanglongbing in East Africa

In East Africa, African citrus greening has caused major yield losses of 25–100% in citrus production, especially for small-scale producers (Ekesi, 2015; Rasowo *et al*., 2019). Indeed, citrus greening has been a persistent constraint to citrus production in Kenya, which has resulted in stagnation in production and low yields, and allowed imports from other countries such as South Africa and Tanzania to meet growing domestic demand (Tschirley *et al*., 2004). For example, in Central province of Kenya, *T. erytreae* ranked highest among the foliar pests, causing 65.1% infestation in young and 50.2% infestation in old orchards (Ekesi, 2015).

Accurate estimation of yield losses due to insect pests and diseases in a perennial crop is difficult. However, HLB disease of citrus in Kenya has been identified as a major limitation (Ministry of Agriculture, 1982). Indeed, citrus losses in Kenyan orchards due to HLB were reported to be over 75% in 2012 (Gitahi *et al*., 2016). Disease management for citrus greening or HLB involves the continual removal of infected trees since there is no cure or resistance. Affected trees provide considerably reduced yields due to continuous fruit drop, tree stunting and dieback, in addition to infected fruits being poor quality and inedible (Bové, 2006; Rasowo *et al*., 2019). Production costs are also increased due to fertilizer costs and more frequent insecticide applications, as well as the cost for removing trees and replanting (Farnsworth *et al*., 2014). Although chemical control has been recommended in response to the insect pest problems on citrus (Beige *et al*., 1984), small-scale farmers often face financial and other socio-economic constraints to uptake (Kilalo *et al.*, 2009). However, over-dependence on chemical pesticides can result in pesticide resistance and is ultimately not economically feasible, sustainable or environmentally appropriate (Gitahi *et al*., 2016). Furthermore, psyllids are persistent vectors of HLB and are throughout their lifetime carriers because of the transovarial transmission of HLB (Kilalo *et al.*, 2009).

Unfortunately, the significant costs in tackling HLB render commercial citrus production unprofitable, resulting in curtailed production or in some cases abandoning citrus production altogether (in Kenya) (Ekesi, 2015; Rwomushana *et al.*, 2017). It has been reported that whole orchards have been lost due to HLB disease (Kenya Agricultural Research Institute, 1991 - in Kilalo *et al.*, 2009). Indeed, pests and diseases, including ACT, false codling moth and HLB, have put the smallholder citrus industry in Kenya in jeopardy, resulting in unemployment and consequently lower living standards and welfare (Gitahi *et al*., 2016).

A phased replacement of infected orchards at low altitudes where citrus greening (*C*Laf) and its vector (*T. erytreae*) do not thrive has been recommended (Tschirley *et al*., 2004). However, the detection of *D. citri* in Kenya, Nigeria and Tanzania has serious implications for citrus production in sub-Saharan Africa (Shimwela *et al.*, 2016; Ajene *et al*., 2020c; Oke, 2020) and is cause for concern as the psyllid can spread fast and rapidly adapt to new geographical areas and ecologies (Shimwela *et al.*, 2016; Rasowo *et al.*, 2019). Although *C*Laf has caused significant decline and stagnation in citriculture in Africa the restriction of its vector *T. erytreae* to high elevations and low temperatures has allowed some degree of management especially in comparison to *C*Las- associated HLB (Aubert, 1987; Graça *et al.*, 2016). The arrival of the vector *D. citri*, globally considered to be the more damaging due to its superior ability to vector HLB, in combination with the presence of *C*Las in Ethiopia and Kenya presents a serious threat to the future of citrus production and subsequently smallholder livelihoods in East Africa.

## 2.3 Potential yield loss to Huanglongbing in East Africa

The development of disease within an individual HLB-infected citrus tree, in relation to tree age and disease load, and the associated impact on yield, fruit quality and long-term tree health is described in Section 1.5 – "Huanglongbing symptoms and crop damage". In addition, Section 2.1 gives an overview of the impacts HLB has had on citrus production worldwide, covering lost production, tree mortality, tree destruction and associated economic costs. Yield losses in trees infected with HLB can be variable, influenced by tree age and disease severity and increasing over time. The international literature can provide insights into HLB-associated citrus yield losses experimentally and at scale, and of what could be expected with widespread establishment of HLB in Eastern Africa.

In São Paulo, Brazil, Bassanezi *et al*. (2011) assessed yield loss to HLB for several sweet orange cultivars in relation to disease severity. It is difficult to establish a quantitative relationship between disease severity and yield for citrus which displays alternate bearing, with trees tending to bear heavy fruit crops one year and little or no crop the following year making forecasting difficult, and with neighbouring plants behaving differently in the same year. Nonetheless, Bassanezi *et al*. (2011) were able to demonstrate that a negative exponential model satisfactorily described the relationship between yield and HLB severity for all varieties of 4–6-year-old trees surveyed in the study. Yield reduction was observed even at low levels of disease severity, contrary to some earlier studies and a high correlation between relative yield and number of fruits indicated that most of the reduction in yield caused by HLB was likely due to early fruit drop or lack of new fruit on diseased branches, rather than only reduction in fruit weight. In trees with 100% symptomatic canopy a relative yield of 14% to 19% was expected. All cultivars appeared to have a similar tolerance to HLB and a single equation was proposed to describe the relative yield–HLB severity relationship for the cultivars assessed: $y = \exp(-1.85x)$ which could be used to predict the economic life of HLB-affected groves in Brazil.

A survey-based study by Singerman and Useche (2016) assessed Florida citrus producers' estimates of HLB infection levels and impact on citrus operations. At this time (approximately 10 years after the arrival of HLB in Florida), the average percentages of HLB-infected acres and HLB-infected trees in a citrus operation were 90% and 80% respectively. Compared to pre-HLB levels, the average percentage of yield loss attributed to HLB was 41%. Central Florida was estimated to experience a 12% greater yield loss than south-west Florida. The study was conducted against a reported backdrop of Florida citrus acreage and yield reductions of 26% and 42% respectively and a production decrease from 242 million to 104.6 million boxes by 2014. In the words of the authors at the time of reporting there was "neither a cure nor an economically viable option for managing HLB-infected trees".

A modelling study by Bassanezi and Bassanezi (2008) assessed the impact of HLB on citrus yield in relation to tree age when infected (Fig. 12). The model estimated yield impacts of HLB in the absence of control, accounting for disease incidence progress, disease severity progress and disease severity–fruit yield relationship according to the age of trees at first symptom onset. Disease incidence and disease severity progression were assumed to be faster in younger trees than older ones due to more frequent and intensive flushes in younger trees attracting a higher population of *D. citri* vectoring HLB allowing higher infection rates, and a smaller canopy size leading to higher relative severity of initial symptoms and faster distribution of the pathogen throughout the tree. Using this approach, the authors estimated that without HLB control measures, São Paulo citrus blocks infected up to 5 years old would suffer a major reduction in yield within 2 to 4 years after the onset of first symptomatic trees. Older citrus blocks would not suffer major yield reduction for 5 to 10 years after the appearance of the first symptomatic trees.

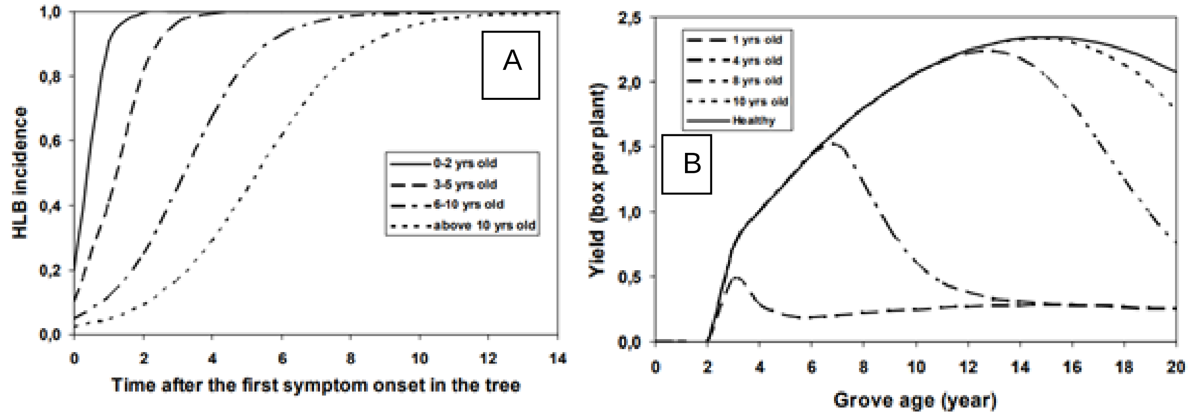


**Fig. 12. A)** Estimated HLB severity curves (proportion of symptomatic canopy area) as function of tree age at first symptom onset in trees without any control measures; **B)** Expected yield curves for healthy citrus groves in São Paulo compared with HLB-infected groves without any disease control, with first HLB symptomatic trees detected at 1, 4, 8 and 10 years old (Bassanezi and Bassanezi, 2008).

These results underline the challenge in convincing growers with older infected trees to destroy them (a matter discussed further below), but also that without HLB control measures it would be extremely challenging to grow economically sustainable new citrus groves using young trees. An innovative strategy implemented in China is to retain citrus in nurseries for 2–3 years before planting out in the field to reduce the attractiveness of the trees to *D. citri*. Bassanezi and Bassanezi (2008) suggest that with additional modification informed by improved knowledge of the disease, this model could be used to generate increasingly accurate and valuable simulations.

The measures implemented to limit citrus yield losses to HLB in Brazil for the benefit of the industry as a whole have brought about challenging societal issues. The coordinated and enforced preventative measures against various citrus diseases in Brazil, which included the frequent elimination of trees and was supported by research institutions and plant protection agencies, facilitated grower acceptance in the early years of the strict recommendations to control HLB (Bové, 2006). As the disease spread, growers' voluntary adherence to and acceptance of the eradication programme and law enforcement diminished and the elimination of trees became voluntary. In orchards with older, fruit- bearing trees exhibiting symptoms but which were still productive, their elimination represented an immediate revenue loss with a consequent faster exit from the business and this caused a resurgence in the disease. In general, from the first appearance of HLB symptoms in 8- to 12-year-old orange trees, it can take 4 to 5 years for the yield to decrease by approximately 60%, compared with non-symptomatic trees (Bassanezi, 2018). Over time, it was evident that elimination, when adopted alone by a grower located in a region with a high HLB incidence, and/or whose orchard was relatively small, had little effect on reducing disease progression within an orchard (Bassanezi *et al.*, 2013a,b).

From 2010–2014, Monzo and Stansly (2017) conducted a large-scale experimental study in orange groves in the south-west Florida citrus-growing area, investigating economic injury levels for *D. citri* control in process oranges from mature trees with high HLB incidence. Trees under the most intensive insecticide application regimes harboured fewest *D. citri* and produced greatest yields, however the more intensive the application regime, the higher the management costs. The authors were able to develop treatment thresholds during the growing season as a function of application costs, juice market prices and *D. citri* densities. The use of conservative thresholds for mature trees with high HLB incidence was expected to help maintain economic viability by reducing excessive insecticide application, leaving room for non-aggressive management approaches such as biological control. For application at a large scale, with citrus of all ages and varying HLB incidence, however, this threshold-based management approach would likely have to integrate effective additional measures for younger trees (Monzo and Stansly, 2017).

The various considerations described above including effective vector management, citrus grove age profile, encouraged/enforced removal and destruction of diseased plants and associated societal challenges must all be considered in HLB action plans and in the immediate response to this damaging disease if yield losses to citrus in Eastern Africa are to be limited.

## 2.4 Estimates of economic loss due to Huanglongbing in East Africa

The discovery of *C*Las in Ethiopia and Kenya (Saponari *et al*., 2010; Ajene *et al*., 2020a), particularly in the presence of its increasingly widespread primary vector, *D. citri*, (Shimwela *et al*., 2016; Rwomushana *et al*. 2017; Rasowo *et al*., 2019; Ajene, 2020c) is understandably a cause of great concern for citrus producers in the region; producers already facing significant pest and disease burdens (Rasowo *et al*., 2019; Wangithi, 2019). The yield losses to Asian citrus greening once widely established can be expected to be significant and a potential threat to ongoing citrus production for resource-poor growers. At this early stage of invasion, however, production losses in citrus due to *C*Las in Eastern Africa are likely to be limited and have received minimal analysis in the literature to date. As infected plants age and symptoms are increasingly expressed, and the disease spreads via its vector(s) to infect citrus more extensively across the region as is probable, the losses to citriculture could be hugely damaging. Producers in higher temperature, lowland areas currently spared losses to the less heat-tolerant African citrus greening disease could now face major losses to the more damaging and heat-tolerant Asian citrus greening, with the majority of citrus in the region now at potential risk from one or both of these diseases (Rwomushana *et al*., 2017; Rasowo *et al*., 2019).

Reporting from regions of the world with a longer history of *C*Las invasion such as Asia, South America and the USA (summarized in Section 2.1) describes a typical sequence of disease progression and impact, namely rapid disease spread via its primary vector, *D. citri*, followed by rising yield losses over time as infected trees become increasingly symptomatic and eventually die, with many trees intentionally destroyed to prevent further spread. In addition to lost production, costs of vector and disease management, replanting and production inputs also increase, while fruit quality is reduced. One country that has broadly avoided this outcome to date is Brazil, the world's largest citrus producer, where a "three-pronged system" was rapidly implemented following detection of HLB in São Paulo State in 2004 (Bové, 2014b). This system involves actively monitoring for HLB, testing and removing infected trees as mandated by the government (Miranda *et al*., 2012); replacing removed trees with healthy trees from nurseries legally required to be covered and insect-proof; and controlling *D. citri* through insecticide treatment of all farmed citrus trees (Bové, 2014b). Management is successful when the percentage of HLB-infected trees on a farm is kept below 1%, therefore, management of HLB must be implemented as quickly as possible following detection of the disease (Bové, 2014b). This has not been the case in Eastern Africa. HLB was first detected in Ethiopia in 2009 (Saponari *et al*.,

2010) and has since become more widespread (Ajene *et al.,* 2020d) and is now accompanied by its primary vector *D. citri* (Ajene *et al*., 2020b), making accelerated spread and increased incidence of citrus infection in the country highly likely. The disease is also now present in Kenya (Ajene *et al.,* 2020a) close to the border with Tanzania, with the key vector *D. citri* present and spreading in both countries (Rwomushana *et al*., 2017). With an immediate and coordinated response across the region of the type demonstrated in Brazil, with buy-in from all citrus growers both smallholder and commercial, there may be a possibility of significantly limiting the spread and impact of HLB in Eastern Africa over the coming years, however, given the response to date and the presence of the disease and primary vector in various agroecological settings in multiple countries, a medium-term outcome closer to that seen in Florida may be more likely and would still require a rapid and extensive response. In Florida, programmes designed to limit the spread and impact of HLB similar to those used in Brazil were rolled out, however, not all growers implemented the suggested measures, which were voluntary and potentially expensive (e.g. destruction of infected citrus trees and coordinated application of specific insecticides) (Singerman and Rogers, 2020). Further measures that have been implemented to manage the disease in Florida include the release of biocontrol agents against *D. citri* and the establishment of Citrus Health Management Areas (CHMA) to provide regional coordination of insecticide sprays targeting the psyllid (Alvarez *et al*., 2016). Analysis of the impacts of HLB in Florida has been ongoing since the disease was first detected and recognised as a threat to the entire citrus industry. Citrus production in the state, a major grower of oranges for juice, is frequently reported in recent literature to have decreased by two thirds or more since 2005 (i.e. ~11–15 years), primarily due to HLB (Monzo and Stansly, 2017; Singerman *et al*., 2018; Trejo-Pech *et al*., 2018; Singerman and Rogers 2020), following production decreases of around 23% over the first five years with the disease (Monzo and Stansly, 2017) up to 49% in a decade (HLB plus other factors) (Alvarez *et al*., 2016). Long term prospects for Florida citrus based on historical yield trends and without significant innovation such as resistant (GM) citrus offer a highly pessimistic outlook (Spreen and Zansler, 2016).

Citrus production systems differ between regions, however, to better understand the scale of risk to Eastern Africa from HLB, it is possible to extrapolate representative levels of loss from other regions suffering the impacts of this disease. Assuming a fairly rigorous response to the disease in Eastern Africa, with vector monitoring and management implemented, removal of infected trees and replanting with certified disease-free stock, but with limitations to coordination and buy-in from diverse growers including many smallholders, the scale of impacts seen in Florida could be realistic with ongoing spread of HLB; possibly more so than those under the highly coordinated and rapid response observed in Brazil, underpinned by legal mandate. Whilst yield losses can vary widely depending on management at the farm scale and the actions of surrounding citrus growers in addition to biotic and abiotic factors, regional production losses over a number of years of coexistence with the disease can give an impression of the potential large-scale, longer-term impacts of HLB to citriculture and factor in various elements in addition to direct yield loss, such as increased disease management costs, greater input requirements, lost trade, tree mortality/destruction and abandonment of production. Additional to lost production will be other important factors such as increased costs to the consumer, lost employment within the sector, and reduced food security of an important source of nutrition. In Table 2, production losses of the scale reported in Florida are extrapolated to Eastern Africa, with near term (5–10 year) and medium-term (10–15 year) annual loss projections.

For Kenya, given the presence of both *D. citri* and now HLB, without rapid containment measures it seems likely that citrus will suffer increasing infection and losses in the coming years to this disease, as has been seen in other parts of the world. Citrus is grown across the country in areas from tropical to subtropical with key production across a band covering parts of coastal, eastern, central and western regions of Kenya over a range of altitudes (Richards *et al*., 2018). Citrus is typically grown in small orchards and backyards by small scale producers with only a few large plantations in Kenya (Richards *et al*., 2018). As a result of ACGD, much of the commercial citrus growing above 800 m.a.s.l. was abandoned in the 1970s, with active production continuing in the lowlands which remain free of the disease (Rwomushana *et al*., 2017). Modelling of environmental suitability for HLB by Ajene *et al*. (2020d) which focuses in detail on Eastern Africa and includes recent records of the disease in the region suggests that the productive western and coastal regions of Kenya show optimum suitability for the disease, and with *D. citri* also present in these areas there is a high risk of spread of HLB to citrus production areas across the central region (HLB and *D. citri* distribution models are discussed further in Section 1.7). Additionally, HLB was detected in citrus trees surveyed by Ajene *et al*. (2020a) close to sea level on the warm Kenyan coast and thriving in higher altitude, cooler regions of Ethiopia more than 2,100 m.a.s.l. The primary vector, *D. citri* was also detected at low altitude sites along the Kenyan coast and has since been detected at altitudes greater than 2,100 m.a.s.l. in Ethiopia (Rwomushana *et al*., 2017; Ajene *et al*., 2020a). Given the apparent ability of HLB to thrive at a range of altitudes, along with its primary host *D. citri* and with potential assisted spread at mid to higher altitudes by the alternate vector *T. erytreae* (Ajene *et al*., 2019; Ajene *et al*., 2020a), and with abandonment of commercial citriculture in many high-altitude areas, 90% of production in Kenya is estimated to be at risk of disease for the purpose of calculating potential citrus production losses to HLB.

In Ethiopia, HLB was first recorded just over a decade ago, but recent studies have found the pathogen to be more widespread and affecting citrus in regions distant from the first detections (Saponari *et al*., 2010; Ajene *et al*., 2020a). Modelling by Ajene *et al*. (2020a) suggests that the majority of citrus production across key districts in the South Nations, Nationalities and People (SNNP) region, Oromia, Amhara and Dire Dawa (Dagnew *et al*., 2014) falls within the area of optimum potential distribution of HLB. Given the recent detection of *D. citri* in the country which is expected to accelerate the proliferation of the disease (Ajene *et al*., 2020a), the broad potential altitudinal and temperature tolerances of the bacterium and its primary vector and the potential role of *T. erytreae* as an additional vector at higher altitudes, it is expected that citrus production across the country faces a significant threat from HLB. As for Kenya, 90% of Ethiopian citrus production is estimated to be at risk from HLB for the purpose of loss calculations.

In Tanzania, *D. citri* was first reported in 2016 from Morogoro (Shimwela *et al*., 2016), with subsequent monitoring revealing an even greater distribution of the psyllid across the key citrus production zones of east to north-east Tanzania including Morogoro, across the coast region to Dar es Salaam and north to Tanga and the Kenyan border (Rwomushana *et al*., 2017). These regions host Tanzania's commercial citrus orchards, mostly at altitudes below 600 m.a.s.l., with backyard citrus trees grown throughout the country at various altitudes (Shimwela *et al*., 2016). Modelling by Ajene *et al*. (2020a) suggests that this important region of citrus production in Tanzania has habitat suitability for HLB ranging from marginal to optimal, with regions in the north, south and west also suitable to varying degrees. Given the presence of the vector *D. citri* throughout the key citrus production areas and the presence of HLB and *D. citri* across the border in Kenya, it is highly likely that HLB will soon reach Tanzania and once present has a high chance of spreading quickly, with subsequent impacts on production. As for Kenya and Ethiopia and given the modelled suitability of key production areas and altitudinal and temperature tolerance observed in HLB and its primary vector, with further risk of spread through *T. erytreae*, 90% of citrus production is estimated to be at risk from HLB for loss calculations.

In Table 2, representative production losses are estimated for a scenario in which HLB is spread via its primary vector *D. citri* amongst the Eastern African countries currently at high risk, with both the vector and disease present (Kenya and Ethiopia) or the vector present and a high risk of the disease arriving imminently (Tanzania). Potential losses are expressed as annual value of lost production based on a scale of production loss comparable (although conservative) to that observed in Florida. Upper and lower bounds of loss are provided for the near-term (5–10 years) and medium term (10–15 years) at 10–30% and 30–60% of current (recent 5-year average) production levels accordingly. Recent producer prices (average 2014–2018 where available, otherwise most recent available average from that range) are used for crop value calculation – citrus prices will likely rise as production decreases and production costs increase, but the benefit of increased prices to the producer will be a deficit to the buyer; in addition, the future citrus price is challenging to predict. Citrus production values by category provided by FAOSTAT vary by country, with some providing a breakdown (e.g. oranges, lemons and limes etc) but others providing grand totals. Therefore, total citrus production values are used and producer price of the primary citrus type (typically oranges) is used for calculation. Where producer price is not available for a country, average price of provided values for other countries assessed is used.

Time lags before significant loss is expected relate to the potentially asymptomatic latent disease period typical of HLB. The increased scale of production loss over time relates to increased tree infection incidence, tree mortality and citrus abandonment, as has been observed in other parts of the world, particularly in Florida and parts of Asia. Production losses over time are significant, but lower than multiple reports for Florida described above; this is in an effort to be realistic in loss scale, but conservative, with rate of disease spread across each country potentially slower than in the high-density citrus production system in Florida, but with management systems potentially less coordinated and proactive in mitigating HLB impacts e.g. due to extensive independent smallholder production with minimal pest control measures applied (Ajene *et al*., 2020b). Key production regions with higher density, high quality and possible commercial citrus are likely to be at risk of rapid spread of HLB via *D. citri*, with smallholder backyard citrus production a potential ongoing source of disease even in the case of proactive insecticide use by commercial growers. Climatic variability may also affect the rate of disease spread and severity, with warm, wet regions at greatest risk (Bové 2014a; Ajene *et al*., 2020b).

For Uganda, neither *D. citri* nor HLB appear yet to be present in the country, despite an earlier report of HLB presence by Kalyebi *et al*. (2015) (see Roberts *et al*., 2017). Significant citrus production is undertaken in the east of the country (Ajene *et al*., 2020a), particularly in Teso region, Busoga region and parts of the central region (Kongai *et al.,* 2018). This mid-altitude (~1,000–1,400 m.a.s.l.) area is expected to be somewhere between marginal to highly suitable for HLB according to modelling by Ajene *et al*. (2020a). Shimwela *et al*. (2016) suggest only the south-east (primarily Lake Victoria) would be highly suitable for the primary vector *D. citri*, however modelling by Narouei-Khandan *et al*. (2016) predicts that much of Uganda including the primary citrus growing areas would be suitable for both *D. citri* and HLB. In addition, *T. erytreae* which is present in Uganda could be a potential additional vector of HLB in the mid to higher-altitude citrus-growing regions should the disease arrive. 90% of citrus production is estimated to be at risk for loss calculations. Citrus production data for Uganda are scarce and highly variable, with no records on FAOSTAT.

Annual citrus production estimates range widely, for example from 10,000 tonnes nationally (Njoroge *et al*., 2009) to 600,000 tonnes in Teso region alone (Dennis, 2013). Kongai *et al*. (2018) report citrus production of 200,000 tonnes in 2009, projected to rise to 360,000 by 2011. In 2015, citrus output in the region was estimated at 769,177 tonnes (Kongai *et al*., 2018). Unfortunately, the primary references for these figures could not be sourced, so the lower of these figures (200,000 tonnes) is used as an estimate for calculating potential losses to Uganda. As neither the primary vector nor disease are yet present in Uganda, HLB losses may not be apparent for some time (if at all if strict quarantine and phytosanitary measures can be implemented), however figures are provided to give an impression of the potential scale of loss to HLB should it arrive, particularly given the presence of HLB and *D. citri* in neighbouring Kenya.

The economic value of potential production losses over time to HLB for the East African countries discussed are detailed in Table 2.

**Table 2** – Economic value of potential citrus production losses to HLB under a scenario of extensive establishment, widespread but poorly coordinated vector management and partial removal of infected citrus disease reservoir.

| Country | Proportion of national production at risk | National production (tonnes) (5-year average 2014 to 2018) | Crop value ($/tonne) (average 2014 to 2018 where available) | Lost annual production value (1000 $) [5–10 years] | | Lost annual production value (1000 $) [10–15 years] | |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | Lower | Upper |
| Ethiopia | 0.9 | 46,082 | 298 | 1,236 | 3,707 | 3,707 | 7,414 |
| Kenya | 0.9 | 216,252 | 250 | 4,873 | 14,618 | 14,618 | 29,237 |
| Tanzania | 0.9 | 418,695 | 272 | 10,258 | 30,774 | 30,774 | 61,547 |
| Uganda | 0.9 | 200,000 | 272 | 4,900 | 14,700 | 14,700 | 29,400 |

Potential citrus production losses to HLB could be substantial in Eastern Africa, particularly for Tanzania, with significant additional impacts on employment, trade and nutrition; increased costs of production and pest management for growers and elevated citrus prices for consumers likely. The loss scenario described would also rely on a level of response to HLB across its current distribution that would include imminent action to remove infected citrus and proactively manage its key vector(s), address the risk of alternative host plants as a disease reservoir and maintain these activities at a significant level in perpetuity until a silver bullet or very effective IPM approach can be established. Without immediate, strong actions as outlined in the recommendations section (Section 5, p67), production losses could be higher still. Immediate, coordinated action, based on a rigorous regional action plan and informed by the activities carried out in areas of the world that continue to manage HLB effectively, as in Brazil, could keep production losses to a minimum. This would require a high level of coordination and buy-in from many stakeholders including countless smallholder producers, with government level oversight and the availability of funds and resources to support the necessary HLB management activities.

Without sustained high level management or development of novel, highly effective and available treatment(s), citrus losses to HLB could be expected to grow further beyond the timeframe in the scenario above, potentially threatening the long-term viability of citriculture in these countries. In addition, climate change may exacerbate losses to HLB, as detailed by Ajene *et al*. (2020a).

For each affected country listed above the losses to HLB would be highly significant, with citrus a growing and valuable commodity in Eastern Africa (Kongai *et al.*, 2018). Losses to major citrus producers in sub-Saharan Africa could be greater still, given the recent discovery of *D. citri* in Nigeria (Oke *et al*., 2020) and with South Africa also vulnerable, particularly to movement of the psyllid south from Tanzania through Mozambique. Key citrus production areas of Nigeria appear highly suitable for HLB, with *D. citri* also expected to thrive here (Narouei-Khandan *et al*., 2016; Shimwela *et al*., 2016; Ajene *et al*., 2020a). Important production areas of South Africa are thought to be suitable for HLB (Narouei-Khandan *et al*., 2016; Ajene *et al*., 2020a), with Shimwela *et al*. (2016) also predicting suitability of much of this area for *D. citri*. Narouei-Khandan *et al*. (2016) suggest some limitation of westward spread of *D.* citri in South Africa under their MaxEnt model, but significant overlap with key production in their SVM model. South Africa has long been preparing for HLB, with extensive direct experience of African citrus greening and close observation of HLB impacts and management in Asia and the Americas informing action plans for the country. Preparedness for HLB in Nigeria is less apparent from the literature, however, the recent arrival of *D. citri* will no doubt prompt accelerated planning to protect the citrus industry. Both countries have the opportunity to mitigate losses to HLB should it arrive and South Africa in particular looks ready to implement measures equivalent to those seen working effectively in Brazil. To demonstrate the potential impact of HLB if high level control were not achieved, the production loss scenario described above for Eastern African countries was applied to South Africa and Nigeria, giving the following production loss estimates:

**South Africa (with an estimated 70% of production at risk)**
5–10 year lost annual production of $48.7–$146.1 million
10–15 year lost annual production of $146.1–$292.3 million

**Nigeria (with an estimated 95% of production at risk)**
5–10 year lost annual production of $102.5–$ 307.6 million
10–15 year lost annual production of $307.6–$615.3 million

These figures demonstrate the huge potential impacts of HLB to major citrus producers in sub-Saharan Africa even under an active management programme and the importance of rigorous, rapid mitigation measures against HLB and its primary vector as implemented by countries that have successfully tackled this damaging disease to date. High phytosanitary standards and exclusion measures to prevent establishment of HLB in each of these countries (and their neighbours) for as long as is feasible will be of significant importance in limiting impacts on citrus.

## 2.5 Huanglongbing and trade

The main means of movement and dispersal of the disease is by vector transmission by the African citrus psyllid, *T. erytreae* and the Asian citrus psyllid, *D. citri*. In terms of trade, the risk of spread is greatest via frequently-traded plant products such as citrus material (budwood, grafted trees, rootstock seedlings) from infected areas which can carry eggs and/or nymphs over longer distances. Indeed, 5th or 6th-instar nymphs, as well as adults born from these nymphs are capable of transmitting the greening/HLB agent to citrus. *Murraya paniculata*, in the Rutaceae family, frequently used as an ornamental bush or hedge, is one of the best hosts of *D. citri* and can carry eggs or nymphs of the vector facilitating its introduction to new regions, and Indian curry leaf tree*, Murraya koeniggii,* is also readily exchanged with Asia and can carry eggs and nymphs to unaffected areas (Rwomushana *et al*., 2017). However, entry on citrus fruit is extremely unlikely for both species (Batool *et al*., 2007). Natural dispersal for both psyllids is likely to be limited to local spread, although Rwomushana *et al*. (2017) suggest *D. citri* can spread considerably from areas in which it is detected (adults can disperse at least 2 km within 12 days and are capable of long distance spread from 90 up to 470 km).

In coastal Kenya there are a wide range of suitable host plants on which *C*Las can successfully multiply and be transmissible (Rwomushana *et al*., 2017). Indeed, increasing regional trade, reservoir host plants and similar climatic patterns could mean *C*Las is more widespread than currently documented in East Africa (Ajene *et al.*, 2020d).

A first point of entry for the vectors likely includes border areas, for instance Rwomushana *et al*. (2017) reported *D. citri* in high numbers in locations bordering Kenya and Tanzania, with a reduction in insects collected moving inland into Kenya. Much of the long-distance movement of vectors and pathogens can be accounted for by movement of infected and infested plants (Shimwela *et al*., 2016). There is a high phytosanitary risk of introducing Liberibacter-infected citrus into regions where the disease may not be present but the vector occurs i.e. via infected citrus budwood or trees (Bassanezi *et al.,* 2020). Regulation of citrus planting materials is urgently needed, for example, in Tanzania, farmers source orange seedlings/trees from their own backyard nurseries or buy them from other farmers with small home nurseries; a potential route for disease spread.

Due to the severity of HLB, EPPO recommended the prohibition of importation of citrus plants for planting and cut branches of citrus from countries where either citrus greening bacterium or either of its vectors occur (EPPO, 2020). *T. erytreae* and *D. citri* are both recommended for regulation as quarantine pests in the European Union, whereby exporting countries may be required to apply mandatory phytosanitary procedures, which results in extra cost to exporters and the National Plant Protection Organizations. At present, none of the bacteria associated with HLB has been found in the EPPO region, although one of its vectors, *T. erytreae*, is present in Spain and Portugal (Siverio *et al*., 2017; EPPO, 2019).

In the Republic of South Africa, where the citrus export market contributes enormously to job creation and economic growth, the Department of Agriculture, Agrarian Reform and Rural Development (DALRRD) has alerted the agricultural community, importers, international travellers, academic institutions and all citizens about the potential risk facing the country, Southern African developing countries (SADC) and the entire continent as regards *C*Las and HLB. The National Plant Protection Organization of South Africa (NPPOZA), within DALRRD, is already developing an early warning system and Steering Committee for this pest in collaboration with the citrus industry. This is in line with the South African Emergency Plant Pest Response Plan. More actors will be addressed in the development of this strategic plan to ensure that all relevant state bodies are included, as well as research organizations, commercial, small-scale and subsistence producers (Larkin, 2021).

# 3 Huanglongbing management

Since its emergence in China nearly 100 years ago, a number of management strategies have been employed in an attempt to mitigate the extensive economic damage caused by HLB worldwide, with the level of reaction largely a function of the disease's status on the invasion continuum. The disease remains widespread in the majority of citrus-producing regions however, and a safe, specific, effective and sustainable treatment for *C*Las has yet to be found. Strategies for control have included insecticides, biocontrol, antimicrobials, cultural techniques and thermotherapy. The lack of breakthroughs in HLB management, despite the significant investments in research, can be attributed to the complexities of the system; notably the host's perennial nature, the lack of resistance in any citrus relative, the difficulty of breeding to produce HLB-resistant cultivars, the pathogen itself, which was only recently successfully cultured in pure form (Ha *et al.,* 2019), the complexity of the vector/bacterium/host interactions and the lack of a good model system have all proved challenging aspects to a sustainable and effective management strategy.

The disease is not easy to recognize in the field and the symptoms can be confused with other diseases or with nutrient deficiencies (Huang *et al*., 1980). A yellowing tree canopy, blotchy mottled leaves and small irregular fruits with aborted seeds provide the best indications of huanglongbing infection however accurate diagnosis is key. A diagnostic protocol for *C*Laf, *C*Lam and *C*Las and for their detection in their psyllid vectors *D. citri* and *T. erytreae* has been published by EPPO (2014). The protocol involves detection based on the disease symptoms and molecular tests (PCR) and it is recognised that sensitive, cost-effective detection of *C*Las infections in citrus and its psyllid vector form a vital part of a vigorous HLB management programme. Currently quantitative polymerase chain reaction (qPCR), along with conventional PCR and DNA sequencing, is used by accredited laboratories for verifying infection. Diagnostic sampling is a challenge however and this is due to the uneven *C*Las spatial and temporal distribution in hosts and vectors, together with the long lag time between inoculation and bacterial detectability (National Academies of Sciences, Engineering, and Medicine, 2018). The early detection of the bacterium in the vector is also essential and can be done considerably before the tree exhibits symptoms, since this can take months to years (Lee *et al*., 2015). Plant inspectorate agencies are advised to adopt regular use of PCR indexing of samples for HLB, to ensure citrus germplasm propagation and shipment of clean citrus planting material (Magomere *et al*., 2009; Shimwela *et al*., 2016).

In the US, substantial financial investment has been given to HLB research for over a decade, in hopes of finding short-, medium-, and long-term solutions; CHMA have been established in Florida, one of the worst hit areas of production, to promote regional coordination of insecticide applications, but their effectiveness is hampered, both by intermittent grower participation and by persistence of vector and pathogen reservoirs in abandoned groves and backyard trees. In these situations, the most effective control strategy has been to remove infected trees in an area and then replant with *C*Las-free trees (Abdullah *et al*., 2009).

Current recommendations are that control of the psyllid vector should be done as soon as its presence is noticed in citrus groves, even in regions free of HLB (McCollum and Baldwin, 2017). In areas where HLB incidence is low, mitigation consists of judicious removal of symptomatic trees, intensive monitoring, chemical and biological control of the insect vector. In Florida, a novel rapid and easy tool has been developed for early detection of citrus greening disease, which can detect and quantify the biomolecule limonin, where elevated content is responsible for the bitter taste in citrus fruits (Saraf *et al*., 2019). Deployment of trained canine teams for detection of *C*Las in commercial and research orchards has also been utilised in California, with dogs detecting infections with great accuracy and discrimination from other pathogens (Gottwald *et al.*, 2020).

With a multi-million-dollar project portfolio, the not-for-profit Citrus Research and Development Foundation (CRDF) was created to supervise HLB research and development efforts in Florida. This included a number of strategies such as interrupting the breeding/feeding of psyllids, vector management, breeding for disease-resistant plants traditionally or through genetic engineering, control of the HLB inoculum and improvement of the host response. Between 2010 and 2017, CRDF invested $124 million in 398 citrus projects, 90% of which were focused on HLB. Support for HLB research has also been provided by the US Department of Agriculture (USDA) and the USDA Agricultural Research Service (ARS), with more than $450 million directed at citrus disease research since 2009 (USDA, 2017). The HLB Multi-Agency Coordination Group was established in 2013 to promote further coordination across federal and state agencies and help provide short term solutions for citrus growers and this too has awarded some $30 million towards HLB research.

The search for a biotechnological answer to control *D. citri* has gone beyond transgenic citrus; for instance, RNA interference has been used to silence genes in *D. citri,* that can alter wing development (El-Shesheny *et al*., 2013; Killiny and Kishk, 2017), increase susceptibility to insecticides (Killiny *et al*., 2014; Kishk *et al*., 2017b), and disrupt sucrose metabolism (Kishk *et al*., 2017a; Santos-Ortega and Killiny, 2018). Biotechnological solutions are considered more environmentally friendly than chemical methods, but some growers and consumers have concerns about transgenic crops. Additionally, many of these experimental approaches are still primarily confined to laboratory and greenhouse settings.

Prevention measures play a pivotal role, particularly in areas where the disease has not yet wreaked widespread havoc. Citrus growers in China have pioneered this strategy and are successfully managing HLB incidence to below 5% over multiple growing seasons, with a three-pronged approach, or "three axes", involving the use of disease-free saplings, removal of infested trees and monitoring/control of vectors. In Brazil, the strategy is much the same, with judicious management of trees and pests allowing growers to stabilize incidence and effectively halt the disease's alarming progression.

Whilst the research in the US and other countries has expanded knowledge of every aspect of HLB, a failsafe management strategy remains elusive. Fighting the epidemic should involve a combination of approaches rather than reliance on a silver bullet. Ultimately, integrating these approaches into coordinated, official, national, regional and area-wide action plans and control strategies, involving growers and citrus/ornamental nurseries and extension workers, should be prioritized before it's too late. Action plans aiming to do this have been developed in countries where eradication and/or containment is still a possibility e.g. in California (Albrecht *et al*., 2020) and Texas in the US, the Caribbean and South Africa (CRI).

In East Africa, the need for area-wide action plans has been recognized and a multi-partnered project "Strengthening Citrus production systems through the introduction of IPM measures for pests and diseases in Kenya and Tanzania (SCIPM)", was implemented and culminated in some key broad, recommendations which will be considered in the final section (S. Faris, ICIPE, pers. comm., 2020).

## 3.1 Chemical control

Worldwide, insecticides have formed a key component of ACP management and when both disease and vector are present, the use of pesticides has often been favoured (Graça and Korsten, 2004). Chemical control is invariably costly however and Boina and Bloomquist (2015) estimated annual costs per hectare of ACP management in citrus to be in the range of \$240 to >\$1000, depending on the type of insecticide used, application frequency and method of application.

The following section provides a summary of the historical literature on chemical control of HLB vectors and many pesticides are either no longer recommended or indeed no longer in use.

Nearly every pesticide available for insect control has been tested against citrus psyllids in Florida (reviewed in Grafton-Cardwell *et al*., 2013; Qureshi *et al*., 2014; Boina and Bloomquist, 2015) and largely consisted of foliar applications of broad-spectrum insecticides and reduced-risk insecticides, as well as soil or trunk applications of systemic insecticides. Psyllid behaviour, population size and life stage at the time of acquisition influence pesticide effectiveness (Pelz-Stelinski *et al*., 2010) and psyllids infected with *C*Las were found to be more susceptible to at least five commonly-applied pesticides than those that were not infected (Tiwari *et al*., 2011).

However, by virtue of its high fecundity and short generation times, *D. citri* can respond rapidly to selection pressures and consequently growers' reliance on the most effective pesticides, applied more frequently and at higher rate, has selected for resistance (Qureshi *et al.*, 2014). Some degree of resistance to key insecticides (organophosphates, carbamates and neonicotinoids) has already been documented in ACP populations in Florida (Tiwari *et al*., 2011; Tiwari *et al*., 2012) and so judicious and rotational application (with different modes of action) was recommended, contingent on actual pest populations and individual or regional assessment of all factors.

As for many cropping systems, there is no universal spray regime to fit every grower. As well as restriction to nationally-registered pesticides, overall cost, efficacy, conservation of beneficial insects and resistance management will all serve to influence the spraying programme. Timing of applications has also been found to be critical for population reduction. New flushes of foliage regulate the dynamics of several citrus pests requiring soft tissues for oviposition and development including *D. citri* and because young trees flush more frequently than mature trees, these need more protection (Qureshi *et al.*, 2014).

Insecticide applications (foliar or soil-applied) in newly-planted or well-established groves was found to significantly delay or lower the incidence and spread of HLB compared with untreated groves. Moreover, a significant reduction in disease spread was observed when the applications were made with an effective insecticide, at a frequency of ≥2 applications per year soon after the incidence of the disease (Chiyaka *et al.*, 2012).

In Florida, systemic neonicotinoid insecticides, such as thiamethoxam (WHO Class II), imidacloprid (WHO Class II), clothianidin (WHO Class II) and chlorantraniliprole (WHO Class U) were used in citrus but their use as soil applications to young trees was rate restricted (Qureshi *et al.*, 2011). Sprays of broad-spectrum insecticides prior to flushing were proven an effective strategy for reducing populations of ACP in Florida, particularly when overwintering adults were targeted (Qureshi and Stansly, 2010). Qureshi *et al.* (2014) investigated 23 products including cyantraniliprole (WHO U), flupyradifurone (WHO II) and sulfoxaflor (WHO II) and found that they provided more than 90% reduction in psyllid populations. With a few exceptions, most foliar sprays were found to suppress adults longer than nymphs and direct effects on nymphs are measurable for only the 2–3 weeks it takes for new shoots to harden and for nymphs to mature to adulthood (Qureshi *et al.*, 2014). Most young nymphs are protected inside the newly-developing unfolded leaves where ACPs oviposit, therefore some avoid contact with insecticide sprays. Consequently, insecticides that leave persistent residues are needed to control nymphs as they emerge from eggs tucked within foliage and to protect trees from incursions of ACP adults. Residual control by foliar insecticides was found to be longer lasting with pyrethroids and thiamethoxam (WHO II) and shortest for insecticides approved for organic production (Qureshi and Stansly, 2020).

These residual populations along with surviving adults are usually enough to initiate a new generation and consequently required follow up treatment (Qureshi *et al.,* 2014). Monitoring the onset and duration of flush periods, together with visual inspection of the flush and trapping to monitor for nymphs and adults, is thought to facilitate timely intervention with insecticide applications and reduction of ACP populations (Boina and Bloomquist, 2015).

Historically in Asia, a range of insecticides, mostly organophosphates and pyrethroids, have been used in very intensive spray programmes to kill nymphs and eggs of the HLB vector on flush growth. In Southeast Asia, control has not been achieved with as many as 35–52 applications of synthetic pesticides a year. These sprays often comprised four or more active ingredients (many of which were highly toxic and illegal). In China, an estimated 10–13 sprays per year were required during flush periods to rehabilitate citrus production in an HLB-infected area (Roistacher, 1996). *D. citri* was controlled at the time using systemic insecticides like dimethoate (WHO II) and other highly toxic pesticides which are banned or heavily restricted by international agreement today (Graça, 1991). In Jiangxi Province in China, protecting the city's $1.5 billion navel orange industry relies on helicopters spraying broad-spectrum insecticides to keep the psyllids in check.

Synchronization of application with or prior to flushing plays an important role, as does the use of sticky cards to monitor *D. citri* in order to time control action (Aubert and Hua, 1990). The timely and appropriate use of insecticides achieved good control of *T. erytreae* in South Africa (Graça, 1991). In Florida, young trees were found to be best protected with soil drenches and trunk injections with neonicotinoids and cyantraniliprole (WHO U) in rotation (Stansly *et al.*, 2012) and thus served as a rotation partner to slow the evolution of insecticide resistance in ACP. However, these soil applications were generally supplemented with foliar applications of broad-spectrum insecticides (Boina and Bloomquist, 2015).

In Brazil's commercial orchards, to prevent secondary infections from diseased trees within an orchard, and primary infections by psyllids from outside the orchard, psyllid control was conducted at biweekly intervals (shorter than the egg-to-adult lifecycle of ACP). Additionally, more frequent insecticide sprays were advocated from bud development to leaf maturity (Cifuentes-Arenas *et al*., 2018) in flushing young trees, freshly pruned or irrigated trees with induced flushing (Hall *et al*., 2016; Cifuentes-Arenas *et al*., 2018). Ultimately, in Brazilian commercial orchards, psyllid control is contingent on tree age, vegetative stage and block position. For nursery trees, drench application of systemic insecticides 1 to 5 days before planting has been recommended. For young trees (up to three years old), three to four applications per year of systemic insecticides by drench or trunk was recommended during the shooting period, plus foliar applications at intervals of 7 to 14 days. For mature trees (> 3 years old), foliar applications at intervals of 7 to 28 days were recommended (more frequently during the shooting period and in blocks at the edge of the farm) (Miranda *et al*., 2017).

Reduced-risk insecticides with novel chemistries and modes of action could be a promising rotational tool in insecticide resistance management programmes for ACP (Boina and Bloomquist, 2015). Lab studies have shown that the IGRs, pyriproxyfen (juvenile hormone mimic) (WHO Class U) and the slightly hazardous buprofezin (WHO Class III) and diflubenzuron (WHO Class III) (both chitin synthesis inhibitors), have promising ovicidal and nymphicidal activities against ACP, as well as adverse effects on reproduction (both fecundity and egg viability) and morphology of adults emerging from treated older nymphs. Controlling nymphs is as important as controlling adults, because pathogen transmission efficiency of infected adults was found to be greater when they acquire the pathogen as nymphs rather than as adults (Tiwari *et al.*, 2012).

Qureshi *et al.* (2014) found that the application of insecticides with horticultural mineral oil (HMO) as adjuvant generally improved their efficacy against ACP in Florida.

A baseline survey of farmers in major citrus-producing counties of Kenya by Gitahi *et al*. (2019) highlighted the lack of knowledge on alternative pest management practices other than pesticides. A study by Constantine *et al. (*2020) also confirmed the low use of biopesticide products in Kenya, alongside high use of conventional chemical pesticides. In Africa, most farmers depend solely on the use of synthetic pesticides to minimize damage and output losses due to the citrus greening pest complex. In the absence of alternative pest management strategies, farmers resort to widespread and unguided use of pesticides, spraying frequently and mixing various pesticide brands in an attempt to make them more effective (Muriithi *et al*., 2016) which is not only expensive, but also often highly toxic with serious negative effects on people and environment. The majority of citrus growers in Machakos and Makueni reported use of synthetic pesticides (91%) (Gitahi *et al*., 2019). Some of the pesticides used are listed as persistent organic pollutants (POPs), leading to rejection of produce in any export markets. In addition, fruit quality is negatively impacted by heavy use of pesticides and the associated depletion of enemies can result in serious outbreaks of minor pests such as citrus red mite, *Panonychus citri*. Furthermore, many farmers base their application of pesticide on the observable damage caused by pests and in the case of HLB, this means the disease can take hold.

In Uganda, dimethoate (Rogor, Perfekthion, Roxion) (WHO Class II) had been recommended for use in citrus (NAADS, 2020). In Kenya, fully registered products for use in citrus include buprofezin (WHO III), bifenthrin (WHO II), imidacloprid (WHO II), carbaryl (WHO II) and diazinon (WHO II) (PCPB, 2018).

While insecticidal control of HLB pests has been shown to significantly increase production, the collateral impact on environmental and public health, side-effects to beneficial arthropod fauna of citrus agroecosystems, risks of resistance and relative costs of insecticide applications compared to the value of the crop make it important to seek and integrate other optimal management options (Monzo and Stansly, 2017). Farmers rely heavily on advice about what products to use, therefore raising awareness and knowledge of those providing advice is essential, as well as understanding farmer's perceptions of alternative controls.

## 3.2 Antimicrobial control

The search for a novel treatment for citrus greening has, to date, relied heavily on modern molecular techniques with a limited number of new chemicals being tested in commercial groves. A number of publications described the use of different compounds against citrus greening disease, including classic antibiotics that are frequently used to treat bacterial infections in animals and humans (Zhang *et al.,* 2014; Hu *et al.*, 2017). Antibiotics have long been used in several agricultural practices and in citrus, the use of trunk-injected or budwood/root-soaked antibiotics has received considerable interest, although it is highly labour intensive. Both methods offered temporary relief of HLB symptoms (1–1.5 years) and in Taiwan, China, Reunion, the Philippines, South Africa and India, trunk injections provided the best results (Graça and Korsten, 2004). Injection of tetracycline into the trunk of affected sweet orange trees in South Africa has resulted in at least partial recovery of the trees. In 2016, the antibiotics streptomycin sulfate, oxytetracycline hydrochloride and oxytetracycline calcium complex were approved for emergency field use in foliar sprays to treat HLB in Florida but after two years the results were inconclusive (Blaustein *et al*., 2017). Populations of residual bacteria that survived the treatment have also been reported to regrow over time (Hu and Wang, 2016). Thus, long-term use of antibiotics may be needed to control HLB, which leads to problems with operational costs and potential adverse environmental effects (Blaustein *et al*., 2017). Phytotoxicity is also well documented in the literature, when tetracycline and oxytetracycline are applied at the concentrations needed for efficacy against *C*Las. In Indonesia, a nation-wide, large-scale tetracycline injection programme failed to give control. Additionally, most antibiotics are temperature sensitive and their efficiency is largely reduced in hot weather. Overall, public concerns about emergence of antibiotic-resistant bacteria and potential side effects on humans are curtailing the use of antibiotics in agriculture and the environment, although all the low- to middle-income WHO regions of the world continue to recommend their use on crop plants (with the exception of Africa) (Taylor and Reeder, 2020).

The race to find a non-antibiotic chemical compound against *C*Las bacterium, which have little to no negative effects on humans is ongoing. Several effective and partially effective non-antibiotic antimicrobial compounds including metals and natural products have been identified and the developments in pure culturing of *C*Las are opening the door to the use of genetics-based methods to understand and mitigate the spread of HLB. A recent discovery of a novel class of heat-stable antimicrobial peptides (SAMPs) which effectively kills the HLB-causing bacterium has been developed from greening-tolerant Australian finger lime and other wild citrus relatives (Huang *et al*., 2021). This natural plant product is reportedly safe to humans, systemic and long lasting and will be further enhanced with proprietary injection technology. Innovative research such as this is needed if an effective, environmentally-friendly treatment is to be found to help with the fight against *D. citri* and HLB.

## 3.3 Botanicals and organic management

The persistent use of broad spectrum, synthetic pesticides in HLB management has led to resistance in citrus psyllids (Tiwari *et al*., 2011) and increased the need for judicious and reduced use of hard chemistry insecticides. Alternative, less environmentally-toxic products such as botanicals have been found to be less harmful to some beneficial insects, including coccinellids and spiders (Walter, 1999; Tang *et al*., 2002) and may have potential for IPM, particularly in habitats where conventional insecticides are not allowed or appropriate such as organic citrus and urban areas.

Petroleum-derived spray oils have been used to control susceptible phytophagous pests and diseases of plants for over a century (Agnello, 2002). These highly refined products are classified as horticultural mineral oils (HMOs) and agricultural mineral oils (AMOs) (Kuhlmann and Jacques, 2002) and are able to kill the insect pests by disturbing their respiratory systems (Liu and Stansly, 1995). These mineral oils offer an economically-attractive and environmentally-safe pest management option which can, in many cases, be accepted for organic production (Stansly and Conner, 2004). Oils have the additional effect of repelling adults from new flush, thereby reducing oviposition and deposits of emulsified HMO on foliage also reduce landing rates, feeding and reproduction on citrus (Ouyang *et al*., 2013). Studies have indicated that effectivity of HMOs is enhanced when combined with improved canopy management (pruning) (Khalid *et al*., 2012) and should coincide with critical stages in order to ensure efficient suppression of insect population (Rae *et al*., 1997). Importantly, these HMOs were found to have no phytotoxicity if used in low enough concentrations (Rae *et al*., 1997) although use of heavy mineral oils was more likely to have deleterious impacts on trees. Growers in organic groves rely mostly on spray oils and botanicals derived from neem and pyrethrum, surfactants, insecticidal soaps and kaolin spray (Qureshi and Stansly, 2020).

Other groups of reduced-risk insecticides that hold potential are botanical insecticides such as neem-based formulations, sucrose octanoate and organosilicone spray adjuvants (Boina and Bloomquist, 2015). All these are effective against nymphs. Insecticides approved for use in organic orchards such as the mineral oils and even bacterial culture or extracts such as *Chromobacterium subtsugae* (Grandevo) (Qureshi *et al*., 2013), whilst capable of comparable levels of suppression to their synthetic alternatives, tend to be short lived. Nonetheless, these are thought to have some use in rotation applications, to reduce selection for insecticide resistance in psyllids, conserve natural enemies, and for application on blooming citrus for which few synthetic products are allowed, and in organic groves that prohibit synthetic products (Qureshi *et al*., 2013). Frequent applications of such insecticides during the growing season have been suggested as being compatible with biological control.

Another promising natural management opportunity could come from the use of volatile chemicals released by plants which not only repel herbivores (Pickett *et al*., 1992; Agrawal and Karban, 1999) but potentially deter their feeding (Jackson *et al*., 1996; Dugravot *et al*., 2003). Interplanting guava, *Psidium guajava* L. with citrus has been reported to reduce populations of *D. citri* and this has been linked to the repellent properties of guava volatiles containing toxic metabolites (Beattie *et al*., 2006; Hall *et al*., 2008; Zaka *et al*., 2010). Guava leaves are known to lower attraction of *D. citri* to orange leaf volatiles (Zaka *et al*., 2010) Additionally, the sulphur compound, dimethyl disulphide (DMDS), produced by guava in response to mechanical foliar injury (Rouseff *et al*., 2008) has been found to confer protection to citrus foliage by impacting on behavioural activity. Application of DMDS in formulation (Qureshi and Stansly, 2020) appeared to cause existing *D. citri* populations to disperse from treated trees and delay ingress into plots. Citrus host plants of *D. citri* did not produce this compound even after mechanical wounding (Rouseff *et al*., 2008). Balandrin *et al*. (1988) found a range of sulphur compounds including di-n-propyl disulphide in neem seeds (*Azadirachta indica*). The Azadirachtin based biopesticide (Azamax™ EC) was also found to be effective against *D. citri* nymphs (Silva Santos *et al*., 2015).

Terpenes found in guava but not citrus also have been reported to have repellent properties (Qureshi and Stansly, 2020) and Alquézar *et al*. (2017) hint at encouraging genetic engineering approaches to modify citrus to emit these naturally repellent chemicals, as was achieved with Arabidopsis plants (Brassicaceae).

Other botanicals and essential oils identified as repellent include crushed garlic chive oil/leaves/plants (*Allium tuberosum*) (Mann *et al*., 2011), as well as tea tree, thyme, rose and lavender oils, with some also reducing attraction to citrus leaves (Mann *et al*., 2012). Fir oil has also exhibited promise for reducing *D. citri* in small field plots (Kuhns *et al.*, 2016). The plant volatile, methyl salicylate, has also been shown to disrupt or confuse *D. citri* (Martini *et al.,* 2016) but has potential to also disrupt parasitoid cues for host detection and therefore use in moderation is suggested (Rodriguez-Saona *et al*., 2011). The use of lures using host plant volatiles may hold promise if they can be optimized to enhance visual surveillance or be integrated in an attract-and-kill strategy whilst drawing psyllids away from target hosts (Qureshi and Stansly, 2020).

In Florida, HLB management strategies also focus on actions to support and prolong tree health and fruit production in infected trees (National Research Council, 2010). Peer-reviewed literature on these practices is scant, but they are based on the premise that stressed trees are more vulnerable to HLB. Through Enhanced Foliar Nutritional Programs, mixtures of readily absorbable foliar nutrients and phytohormones are applied as sprays and aim to compensate for nutrient losses caused by the blockage of conducting tissues in diseased trees. Anecdotal reports of increased yields and improved health of trees have been reported by growers (Muraro, 2012) but researchers have raised concerns that reducing or ceasing efforts to control the ACP in favour of nurturing infected trees places the two management approaches in conflict with one another (Gottwald *et al*., 2012) and critically could potentially lead to the proliferation of the disease as the inoculum remains in the trees. Ultimately and despite the potential for short-term tree health improvement and reduced HLB impacts on fruit yield, these costly approaches are not curative nor sustainable (Rouse *et al.,* 2017).

As well as enhanced nutrition by foliar sprays, regulation of soil pH to enhance nutrient uptake, and precision irrigation based on soil moisture sensing and needs of HLB-affected trees approaches have also been adopted in sophisticated, multifaceted approaches to water and nutrient supplementation in high-intensity production systems (Roka et al., 2009; Zheng et al., 2018).

Compounds perceived to activate systemic acquired resistance pathways in plants (such as salicylic acid) to increase tree defence responses have also been used (Masuoka *et al*., 2011; Baldwin *et al*., 2012). Plant growth regulators have also been tested, unsuccessfully, to reduce HLB-associated fruit drop (Albrigo and Stover, 2015).

Tomaseto *et al.* (2019) also reported that the use of trap crops on the border of citrus orchards, such as orange jasmine (*Murraya paniculata*), treated with insecticides to attract and kill *D. citri* before settling, could prove an effective strategy if implemented close to citrus tree planting. Further integration with other tactics (e.g. kaolin), as a push-and-pull strategy, could further decrease infestation rates inside citrus orchards.

Gitahi *et al*. (2019) found citrus farmers from Machakos and Makueni counties in Kenya used intercropping (1.2%), plant-based pesticides (6%) and irrigation (4%) practices to manage citrus pests.

Other methods to prevent psyllids from settling or feeding on leaves have also been investigated. Products formulated with kaolin clay have been shown to reduce adult and psyllid numbers on flush, disrupting plant-finding ability and dispersal (Miranda *et al.,* 2018).

## 3.4 Resistant varieties

HLB's intractability is in part due to the lack of natural resistance in any citrus relative and the difficulty of breeding to produce HLB-resistant cultivars (Bové, 2006; Graça *et al*., 2016; Miles *et al*., 2017). It is also thought that the association between *Citrus* and liberibacters is a recent one and as such, too short for the trees to have built up resistance to the bacterium (National Research Council, 2010). R&D efforts in the US have focused on conventional resistance breeding programmes of new rootstock and scion varieties to provide resistance/tolerance to *C*Las, whilst also funding projects into genetically engineered (GE) resistance (National Academies of Sciences, Engineering, and Medicine, 2018). The genetic complexity of the *Citrus* genus results in high levels of heterozygosity, and consequently many generations of breeding and selection are required to develop new cultivars which possess the combinations of genes required for the commercial fresh or processing markets (e.g. fruit and juice quality, productivity, biotic and abiotic stress resistance traits) (National Academies of Sciences, Engineering, and Medicine, 2018). Furthermore, seed-borne breeding programmes are challenged by the 3–7 years of tree juvenility, along with the large areas of land required to grow and evaluate trees.

While a great deal of breeding has been carried out in the United States and other major citrus-growing areas, most of the commercial cultivars currently grown were selected in the 19th century (National Academies of Sciences, Engineering, and Medicine, 2018). Genetic improvement through engineering allows some of these challenges to be bypassed, targeting improvement of existing cultivars, avoiding random assortment of genes and the loss of existing genetic integrity from the desired cultivar. Traditionally, citrus cultivars are propagated vegetatively by grafting to maintain the integrity of the desired cultivar. Rootstocks are selected for disease and nematode resistance, soil adaptation, resistance to flooding and drought, cold hardiness, and effects on tree size (Castle *et al*., 2016).

Whilst there are currently no commercial citrus cultivars, varieties or scion-rootstock grafting combinations with innate resistance to *C*Las infection (Graça *et al.*, 2016) some commercial varieties such as lemon [*C. limon* (L.) Burm. F.] and Persian lime (*Citrus latifolia*), along with US-897 rootstock (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) and the "LB8-9" Sugar Belle® mandarin hybrid (SB; "Clementine" mandarin × "Minneola" tangelo) (Gmitter *et al*., 2010) show apparent HLB tolerance under natural HLB-endemic conditions in Florida (Deng *et al.,* 2019), whilst SB mandarin and "Bearss" lemon trees also maintain vigorous growth and fruit yield compared to many sensitive sibling mandarin cultivars; HLB-tolerant citrus relatives also include Australian finger lime (*Microcitrus australasica*), Australian desert lime (*Eremocitrus glauca*), Hawaiian mock orange (*M. paniculata*) and Khasi papeda (*Citrus latipes*) (Huang *et al.,* 2021). Orange, mandarin and lemon represent three different kinds of citrus from the taxonomic point of view (Wu *et al.,* 2014). Despite representing genetically diverse citrus species, Deng *et al*. (2019) report evidence of shared anatomical responses underlying HLB-tolerance in "Bearss" lemon and LB8-9 Sugar Belle® mandarin; decreased phloem destruction and increased replacement phloem generation was found in field grown trees naturally exposed to HLB and this anatomical insight provides some potential for incorporation into breeding programmes to bridge production as well as develop new approaches to combat HLB in the future.

Genetic engineering can potentially circumvent some of the challenges inherent in citrus breeding programmes and research into gene editing and silencing of bacterial proteins and insect salivary effectors, which contribute to the disease spread may hold valuable potential. Complete control of HLB may not be feasible until plants expressing high levels of resistance to the vector and/or disease are available or can be bred or engineered.

## 3.5 Biological control

Indigenous generalist predators have always been an important component of citrus insect pest management (McCoy, 1985). In Florida and California, ladybirds (Coleoptera: Coccinellidae), syrphid flies (Diptera: Syrphidae), lacewings (Neuroptera: Chrysopidae), and predatory mites (Acari) (Michaud, 2004; Michaud and Olsen, 2004; Qureshi and Stansly, 2007; Juan-Blasco *et al*., 2012; Kistner *et al*., 2016b) are all common predators of citrus psyllids and have been found to play a vital role in regulating populations of *D. citri* (Berg *et al.*, 1992; Qureshi and Stansly, 2009). The extent of control provided by each individual taxon remains largely unknown, though coccinellids seem to provide the most ACP control in Florida (Michaud, 2004; Qureshi and Stansly, 2007), whereas in California, syrphid (*Allograpta* species) and lacewing (*Chrysoperla* species) larvae feeding on ACP nymphs have a collective predation rate inflicting 86% mortality (Kistner *et al*., 2016b). Work from Pakistan indicates spiders are not important ACP predators in unsprayed citrus orchards (Vetter *et al*., 2013).

In their native range, among the most effective natural enemies of HLB vectors are Hymenopteran parasitoids from the genus *Tamarixia* (Hoddle and Pandey, 2014). The complex of parasitoids was comprehensively studied in the 1960s–1970s in South Africa and Swaziland and subsequently in Cameroon some 30 years later (Tamesse and Messi, 2002; Pérez-Rodríguez *et al.*, 2019). In areas where insecticides are not sprayed in South Africa, such as abandoned or experimental orchards and gardens, parasitoids were found to be important biotic regulators of *T. erytreae*. The two main primary parasitoids of *T. erytreae* in South Africa are the ectoparasitoid *Tamarixia dryi* (Waterston) (Hymenoptera: Eulophidae) and the endoparasitoid *Psyllaephagus pulvinatus* (Waterston) (Hymenoptera: Encyrtidae) (Pérez-Rodríguez *et al*., 2019). Both are solitary koinobiont parasitoids (in other words they parasitize earlier host stages and allow host activity to continue feeding and growing during parasitism). A third, new parasitoid species from the genus *Tamarixia* was also recorded by Pérez-Rodríguez *et al*. (2019) in South Africa. These primary parasitoids are themselves frequently attacked by a complex of hyperparasitoids which significantly reduce their impact (Berg and Greenland, 2000). *Aphidencyrtus cassatus* is considered the most abundant (Aubert, 1987). Growers in Florida have released the convergent ladybird *Hippodamia convergens* Guérin-Méneville from California; however, it is still not common there in citrus (Qureshi and Stansly, 2011).

The parasitoid *T. dryi* was successfully used as a classical biological agent against *T. erytreae* in the islands of La Réunion and Mauritius (Etienne and Aubert, 1980; Aubert and Quilici, 1988). In these islands, both HLB psyllid vectors *T. erytreae* and *D. citri* coexisted, as well as *C*Las and *C*laf but only the control of *T. erytreae* by *T. dryi* was successful (Pérez-Rodríguez *et al*., 2019). Almost complete elimination of HLB was achieved in Réunion through a combination of biocontrol (taking care not to introduce hyperparasites in the process), removal of infected trees and replanting with bacteria-free material. The lack of success controlling *D. citri* is linked to a number of factors, including size of population, availability of alternate host plant for *D. citri* and host psyllid for *T. dryi,* as well as climate suitability (Halbert and Manjunath, 2004).

Two parasitoids from the Indian subcontinent, the endoparasitoid *Diaphorencyrtis aligarhensis* (Shafee, Alam and Agaral) (Encyrtidae) and the ectoparasitoid *Tamarixia radiata* (Waterston) (Eulophidae) have also been used for classical biocontrol of *D. citri*. Both kill their hosts through a combination of parasitism and host feeding (Rohrig *et al*., 2011). *D. aligarhensis* attacks second through fourth ACP instars (Skelley and Hoy, 2004; Rohrig *et al*., 2011) and a single female can kill up to 280 nymphs in her lifetime (Chien *et al*., 1995). *T. radiata is* an efficient idiobiont (lives outside the host whilst preventing development) of ACP nymphs, inflicting mortality of >90% of presented nymphs (Skelley and Hoy, 2004) and a single female can kill up to 500 nymphs in her lifetime (Chien *et al*., 1995).

These two parasitoids have been released in Mauritius, Réunion Island, and Florida and California (US) (Michaud, 2002; Hoddle, 2012). *T. radiata* was also released in Taiwan and Guadeloupe. Dramatic success was achieved by *T. radiata* in Réunion Island and Guadeloupe (Hall, 2008). In Florida, *T. radiata* established successfully but control was variable, with reduction in psyllid populations ranging from 4 to 70% and impact limited by local predators (Michaud, 2004) or significant levels of parasitism (20–56%) (Qureshi *et al*., 2009). In California, *T. radiata* established (Hoddle, 2012), and, acting with local generalist predators, it reduced the net reproductive rate of *D. citri* by 55–95% (Kistner *et al*., 2016a). *D. aligarhensis* has been recovered in California (as of 2017) in the majority of release sites, with parasitism ranging from 0.2–34.5% but it failed to establish in Florida. More time is needed to evaluate the impact of the parasitoid on pest populations in California. *T. radiata* and its psyllid host have also been inadvertently introduced into Texas, Puerto Rico, Venezuela, and possibly other areas, most likely through the movement of infested host plant material from where they were previously associated.

In Florida, generalist predators such as coccinellids can cause >95% mortality of ACP parasitized by *T. radiata* (Michaud, 2004) and this can cause parasitism estimates in the field to be underestimated (Michaud, 2004; Qureshi and Stansly, 2009). In California, invasive ants such as *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) which form mutualisms with honeydew producing Hemiptera such as ACP, provide active defence from predators and parasitoids, and can considerably reduce the efficacy of biological control agents in citrus orchards (Vega and Rust, 2001). Whilst undeniably useful in minimizing immigration into commercial orchards from urban trees (backyard citrus) and abandoned groves, the level of control offered by indigenous and imported natural enemies is not adequate as a stand-alone practice in commercial orchards because of ingress of psyllids from abandoned orchards (Lewis-Rosenblum *et al*., 2015). Although the combined effects of these natural enemies have failed to curb the spread of HLB in Florida, conservation of these and other beneficial arthropods is essential for effective citrus pest management in other parts of the US and elsewhere. Diniz *et al.* (2020) reported that the release of parasitoids in areas outside the managed groves, such as abandoned orchards or residential trees, has the potential to maximize actions for *D. citri* control, contributing to the reduction of psyllid ingress into commercial areas.

The rotational pesticide application patterns of many commercial citrus groves, while critical to mitigate against resistance development by the psyllid, will be detrimental to any potential benefits biocontrol agents confer. Additionally, the parasitoid *T. radiata* attacks nymphs (Skelley and Hoy, 2004), but trees can become infected by adult feeding well before any subsequent nymphs develop to an acceptable stage for parasitoid oviposition. As a result, the primary long-term benefit of psyllid suppression by *T. radiata* in commercial orchards would be to reduce the production of infected adults developing from nymphs; whilst this has benefits, a reduced adult population would not eliminate the loss of the infected trees or the eventual spread of the pathogen. Garcia *et al.* (2019) have developed an adaptable computational index to identify suitable areas for biocontrol and successfully tested the *D. citri* – *T. radiata* system using Brazil as an example. Field data indicated that this index is reliable for mapping "suitable" regions that can potentially support biological control programmes.

Entomopathogenic fungi used as biopesticides may also play an important role for regulation of ACP and provide an eco-friendly management strategy for the integrated management of *D. citri* but the use of insect pathogenic fungal sprays to control psyllids has not been reported (Hall, 2008). The entomopathogenic fungi *Cladosporium* sp. nr. *oxysporum* and *Capnodium citri* have been considered important mortality factors for *D. citri* in Réunion Island (Aubert, 1987). Nymphal mortality rates of 60–70% occurred where minimum daily relative humidity exceeded about 88% (Aubert, 1987). The fungus *Hirsutella citriformis* was reported to be common in Guadeloupe during periods when humidity was greater than 80% (Étienne *et al*., 2001). In Florida, dead *D. citri* adults infected by *H. citriformis* (Meyer *et al*., 2007) have been observed from mid-summer through winter, mainly in larger trees (Hall *et al*., 2008) whose microhabitat the fungus may favour. The fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) has been reported to kill *D. citri* in Florida (Meyer *et al*., 2008).

Virulent fungal strains of *Cordyceps fumosorosea* (syn. *Isaria fumosorosea*)*, Beauveria bassiana and Metarhizium anisopliae* have been assessed in laboratory and field conditions (Majeed *et al*., 2017; Saldarriaga *et al.*, 2017), the first two achieving high adult mortality, particularly with high relative humidity and rainfall. Ibarra-Cortes *et al*. (2018) ascertained the fatality rate of *C. fumosorosea*, *B. bassiana* and *M. anisopliae* to be 28%, 55%, and 43%, respectively, to *D. citri* adults, and 47%, 69%, and 100%, respectively, to the nymphs when applying spore suspensions of the individual fungi under laboratory conditions. A study by Avery *et al.* (2011) documented that adult psyllids infected by *I. fumosorosea* have reduced feeding, which could potentially reduce the spread of huanglongbing. Glasshouse experiments with a new strain of entomopathogenic fungus, *Cordyceps javanica* have also shown potential to have high infection rates (Ou, 2018).

Bacterial-based biocontrol is another eco-friendly control method generally compatible with organic production. Citrus-inhabiting bacteria with antimicrobial properties have been assessed (Riera *et al*., 2017) and found to slow the disease progression but only if applications were made during very early infection. Beneficial microbes may suppress infection and slow symptom development by triggering host plant defence responses (Wang *et al*., 2017). Because biocontrol efforts can be hampered by low microbial survival, several candidate beneficial bacteria are being analysed for antimicrobial compounds that could be extracted for concentrated application (Wang *et al*., 2017).

The potential efficacy of entomopathogens will include ambient air temperatures and relative humidity within the citrus grove, air circulation within the canopy, accurate spray equipment and thorough coverage of the foliage during spray applications. High levels of rainfall and UV-exposure are expected to limit the persistence of fungi (Ignoffo, 1992). Sprays should be timed early against developing populations and repeated or integrated with other control methods. Avery *et al*. (2011) showed that infected psyllids mostly stop feeding within 24 h of infection, suggesting they might not actually transmit disease during this time which might mitigate against slower kill rates compared to insecticides.

In Machakos and Machueni counties in Kenya, 8.6% of citrus farmers reportedly use biocontrol against pests and diseases in their farms (Gitahi *et al*., 2019).

In summary, biological control holds a great deal of potential and has proven itself to be an important tool for reducing the incidence of the disease on commercial farms; in particular, reducing the production of infected adults developing from nymphs in peripheral reservoirs to limit ingress outside the managed groves, such as abandoned orchards or residential trees has its benefits. However, reduced adult populations would not eliminate the loss of infected trees nor the eventual spread of the pathogen and so biocontrol needs to be integrated with other management tools for control to be successful.

## 3.6 Good Agricultural Practice

Good agricultural practices for citrus include appropriate site selection, adequate plant population (110 trees per acre), use of clean planting and grafting stock (obtained either from disease-indexed nursery stock, or from disease-free areas), irrigation, the application of organic and inorganic fertilizers, regular pruning of overcrowded canopies (best done with mechanical pruning saws or secateurs as citrus is a hardwood), regular weed control, judicious and timely use of pesticides, good farm sanitation (removal of infested trees ideally when psyllid populations are low to prevent disturbance and spread (Buitendag and von Broembsen, 1993) and harvesting with care to help maximise yield.

According to Ghana's Ministry of Food and Agriculture (MoFA) and GIZ, the German Development Agency, citrus producers in Ghana (where citrus is planted over a larger area than any other horticultural crop) can expect to double their yields, when following these guidelines (GIZ, 2015).

Monitoring and surveillance of ACP in citrus groves and rutaceous trees elsewhere is vital for psyllid prevention. Visual inspection of eggs and nymphs on flush shoots (Sétamou *et al*., 2008), monitoring of adult populations using yellow or lime-green sticky traps (Hall and Hentz, 2010), stem tap sampling (Stansly *et al*., 2010), vacuum sampling (Thomas, 2012), and/or sweep nets (Stansly *et al*., 2010) can all be employed. To improve trapping efficiency, scientists have tested mating disruptors (Mankin *et al*., 2016), odorants released by citrus plants that attract ACP (Coutinho-Abreu *et al*., 2014), and sex pheromones (Zanardi *et al*., 2018). In addition, a reported strong edge effect in *D. citri* distribution in groves, has informed the efficient setup of traps to monitor ACP (Gottwald, 2010). Significant progress has also been made toward reducing psyllid access to young citrus by planting seedlings on beds covered with metalized polyethylene mulch. The mulch should be at least 3 mm thick, with a clear, UV-stabilized coating to protect the reflective surface (Croxton and Stansly, 2014).

The benefits reaped from following recommended practices can be all too easily offset however, by the increased costs entailed and this may prohibit adoption by many small-scale farmers. Despite a paucity of peer-reviewed literature to validate these approaches, it is thought that vulnerability to HLB can be somewhat mitigated by enhancing tree health, be it through good husbandry, orchard sanitation, use of reflective mulch to repel ACP and/or manipulation of nutrition and irrigation.

In the US and China where HLB has had devastating impacts, some of the innovative strategies used to support the management of Asian psyllid infestations have been: (i) the use of wind breaks (natural or artificial) on the windward edges of groves to reduce incursion; (ii) replacement (reset) planting of young trees within mixed, mature groves (since large block plantings create the perfect microclimate for the vector); (iii) delayed transplanting of seedlings into fields until they are 2–3 years old to counter the increased susceptibility and rapid decline of young trees following HLB infection (Stansly and Rogers, 2006); and (iv) orientation of newly-planted citrus rows along the north–south axis (Martini *et al*., 2015). Individual protective tree covers, particularly for young trees, have also been effective by physically excluding psyllids from trees as well as the use of screened greenhouses (530 x 530-micron mesh) (Stansly and Rogers, 2006) or greenhouses with positive pressure ventilation systems.

Almost every country facing the threat of citrus disease has adopted a certification programme to rebuild or preserve its citrus industry (Vapnek, 2009). Underpinned by quarantine, clean stock and track and trace distribution, these programmes, when mandatory, create precise requirements and obligations to prevent introduction and spread and facilitate detection and control. In Kenya and Tanzania, there is no citrus certification scheme and therefore no assurance that nursery-sourced planting material is greening disease-free. In Tanzania, young trees used for replacing old or diseased trees as well as trees required for expanding orchards are either produced by farmers themselves (66.7%) or purchased from other farmers that have small home nurseries (23.3%) or obtained through wild rootstock (10%) (Izamuhaye, 2008) and there is no certified source of plant material. This is also the case in Kenya, meaning there is no assurance that material derived from nurseries will be disease-free (Kyalo *et al.*, 2018).

Rasowo *et al*. (2019) reported that around 59% of the 105 farms visited in Kenya and Tanzania had unhealthy citrus trees, with disease-free citrus only recorded in farms where intensive management practices were employed. Most farmers (85%) grew grafted citrus seedlings obtained from nurseries along the roadsides. Half the farmers occasionally treated their citrus plants with pesticides if and when they saw pests, but only 14% of the farmers used fertilizers. Farmers tended to rely on their own experience (47%) or advice from agricultural commercial retailers (40%), with just a few (13%) taking advice from government extension officers. Over 90% of farmers could not recognize HLB/ACG symptoms or the psyllid vector.

Indigenous Technical Knowledge in Kenya relies on availability of local materials and human resources; some the practices include use of wood ash, kerosene, table salt, lime, cow urine and cow dung but according to Kilalo *et al*. (2009), insect pest management practices by citrus farmers in Kenya are inadequate to deal with the pest and diseases situations within farms with use of synthetic pesticides remaining the most prevalent among citrus fruits farmers. Very few surveyed farmers in Kenya's Machakos and Machueni counties knew that they could remove affected plant parts and plant disease free materials to control ACT and greening disease for instance (Gitahi *et al*., 2019).

In summary, improving the orchard environment through good management and vigilance plays a very big role in mitigating HLB. The layout and design should ensure good ventilation and exposure to sunlight, good soil management including efficient fertilization programmes, inoculum removal and good quality and disease-free planting material. While these practices may not keep the trees free of greening and virus diseases indefinitely, they will prolong their useful lifespan and keep the trees productive for some years even after they have been infected (FFTC, 2003).

## 3.7 IPM

The rationalization of pesticide use is a cornerstone of IPM and its economic and environmental advantages in citrus groves, over a purely chemical approach, have been demonstrated (Hattingh, 1994). The citrus agro-system lends itself well to IPM since citrus trees are long-lived and not disrupted by frequent ploughing or planting. Studies in South Africa, California and Australia have all reported cost savings linked to reduced input costs and higher prices for pesticide-free fruit (Urquhart, 1999). Economic thresholds based on economic injury levels are key to integrated pest management systems, but these are hard to employ when the pest is a disease vector, because of uncertainty as to the proportion of potential vectors capable of transmitting the pathogen. Furthermore, the damage potential of the disease is to some extent a function of the age of the plant when infected (Stansly and Qureshi, 2020). Nonetheless, to implement IPM of *D. citri*, it is necessary to monitor adult and nymphal populations since information collected can be used to make decisions about spray applications. Effectiveness of ACP spray programmes can also be evaluated by monitoring population trends using visual inspections as well as methods such as tap-sampling (hitting a branch with a blunt object to dislodge adults), sticky traps and sweep-net sampling, which have all been found to have similar sensitivity per sampling effort (Monzo and Stansly, 2020). Stem taps have the advantage of rapid data processing time and less sampling effort required to attain similar precision levels at all but the lowest ACP densities and are thus recommended for large-scale ACP monitoring plans (Monzo and Stansly, 2020), whilst sticky traps are advisable for early ACP detection and to monitor young plantations where stem tapping is not efficient. Adult visual sampling also provides a cheap alternative to stem tapping in cases of low ACP infestation levels and can record nymphs on expanding flush shoots (Flores *et al*., 2009; Hall *et al*., 2010) as well as adults although data consistency may be compromised by scouting ability and expertise so adequate training is essential. However, because symptoms are irregularly distributed and *C*Las has been found in higher concentrations away from the trunk, visual surveys need to view the aerial parts of the canopy (Teixeira *et al*., 2008).

Due to the fact that ACP exhibits strong border effect in its niche occupation in groves, recommendations are made to monitor its populations on trees along grove borders (Sétamou and Bartels, 2015). Moreira *et al*. (2019) also observed lower cumulative HLB incidence in citrus plots with higher tree densities.

The challenges of adoption of IPM, such as increased labour and monitoring costs and reduced production during transition periods, can be considerable. Fenemore and Norton (1985) found that high pesticide use was more likely in high value fruit crops to manage the risk of producing low quality product and due to economies of scale achieved by mixing groups of pesticides (e.g. fungicides and insecticides) when no viable alternatives to a single disease problem existed. Gitahi *et al*. (2019) found IPM was only practiced on small scale by farmers but that there was high demand and willingness to pay for an IPM package as a substitute to conventional pesticide use. To encourage the adoption of IPM technologies by farmers, government should consider providing some form of support for the transition period. In order to ensure benefits arising from IPM are distributed more equitably, particular attention should be paid to providing extension support, as well as management and technical training, for emerging farmers, including women to promote awareness of the possible cost effectiveness of an IPM approach, as well as health and safety benefits (Urquhart, 1999).

In South Africa, *C*Laf was responsible for causing severe losses up until the late 1970s (Buitendag and von Broembsen, 1993), however the incidence of HLB is now maintained at economically acceptable levels through integrated management strategies involving inoculum removal and vector control (Pretorius and van Vuuren, 2006). Furthermore, the HLB management experiences in Brazil demonstrates that the *C*Las disease can also be managed if the preventive management practices of inoculum reduction and vector control are applied at a regional scale (Bassanezi *et al*., 2020).

Whilst both biological and chemical control strategies are important components of an IPM programme for ACP, biocontrol agents are slow to suppress populations compared to insecticides and may not be effective in preventing HLB spread. Therefore, once the HLB disease is detected and/or becomes widespread in an area, the chemical control option has advantages, mitigating vector and disease spread, with barriers, repellents and sanitary measures used to further reduce psyllids to very low levels. Reduced-risk insecticides with systemic action (applied to the soil) have been used as alternatives to broad-spectrum insecticides in IPM for ACP in the US (Boina and Bloomquist, 2015) to try to preserve the diversity of biological control agents and maintain ecological stability. Research has shown that 96% of adult psyllids fly at heights less than 7 ft, and >99% of ACP fly at less than 9 ft. Fencing with ACP-impermeable mesh on the perimeters of an orchard, which extends above these heights, has been shown to substantially reduce psyllid introduction into orchards (Sétamou *et al*., 2018). Live windbreaks at borders, while potentially not as effective as fencing, have also been shown to reduce psyllid numbers in orchards (Martini *et al*., 2015).

The rapid movement of the bacterium to the roots (Raiol Júnior *et al*., 2017) is the main cause for the failure of methods such as pruning and thermotherapy. Foliar nutritional sprays, resistance inducers, phytohormones and antibiotic treatments have limited effects on HLB bacteria titres, maintenance of tree health, yield and fruit quality. Foliar nutritional sprays only correct mineral deficiencies in the healthy parts of diseased trees but do not reduce HLB symptoms of leaf mottle and lopsided fruit (Vashisth and Livingston, 2019; Bassanezi *et al*., 2020). Finding the best combinations of control strategies suited to different environmental and growing conditions, vector and pathogen pressure, tree cultivars and tree health would benefit growers where HLB is not yet a chronic problem but soon could be, especially if HLB infection is not detected quickly and if there is reluctance to remove inoculum sources. Effective quarantine measures are essential to prevent the introduction of the HLB organism or the vector, bearing in mind the possibility that the HLB-carrying *D. citri* vector could be introduced 'naturally' or through alternative hosts such as *Murraya* spp. (Graça and Korsten, 2004).

**Summary and recommendations on control methods**

It is widely acknowledged that the best strategy against HLB is to prevent the introduction and establishment rather than to cure the HLB disease. Su *et al*. (1986) were the first to define the three-part management strategy that is now recommended around the world. Their programme included production of clean plants (insect proof nurseries and production and trade of clean citrus material), comprehensive psyllid control and elimination of known inoculum. Where the disease has not yet been detected, the recommendations include quarantine measures against both vector and disease to mitigate human movement of infected citrus material (grafted trees, seedlings, budwood) and rutaceous hosts which can undermine any management efforts if neglected, as well as early detection and diagnosis of HLB and its vectors, removal of inoculum, and scouting for and eradication of citrus psyllids, once identified, using focused and appropriate control methods. In areas where ACP is still absent or scattered, prevention policies to avoid its establishment are called for.

Systematic, delimiting surveys across the region are required to establish the exact distribution of HLB in the East African region and bordering southern areas and to validate occurrence reports. Furthermore, data quantifying the range and number of host plants and the extent to which they have been infected (hot and low spot areas), as well as the prevalence of ACP and the proportion of the population that is infected is needed. This would involve sending samples to taxonomists and research labs for taxonomic/PCR confirmation, which in turn would need to be underpinned with surveillance monitoring/trapping training). Infected psyllids can provide early warning of the presence of *C*Las, prior to symptom development. Track and trace system for trees is also needed to qualify the potentially wider distribution of infected material and to evaluate the prospects for eradication and/or containment for the region given the localization of HLB-positive sites identified by Ajene *et al.* (2020a) in Kenya (Gebeyehu et al., 2020).

Awareness raising should also form a crucial aspect of any action plan, with mass campaigns (media, stakeholder workshops including policy makers, citrus industry, regulators, farmers, extensionists, quarantine personnel etc), training opportunities to build capacity and plant health rallies to disseminate information in local languages. Table 3 provides a synthesis of recommendations on surveillance and management for African countries.

There is a closing window of opportunity to eradicate/contain the disease in East Africa. Much research has been conducted into HLB and its vector around the world and lessons can be learned from the action plans and management programmes which have been or are being implemented in many countries. Whilst it is acknowledged that the best management options may be difficult to implement in different countries and will be influenced by the technology available and existing resources, a multifaceted, integrated management programme, with clearly defined roles and responsibilities between sectors, is essential and coordination within the East African region must be promoted in order to maximise and share experience and resources for the management of HLB. It is important to acknowledge that grower choices and behaviours are fully intertwined with many different issues and outcomes. Individual grower needs and scale of production, and also that of their neighbours, all influence disease spread, vector population dynamics and areawide adoption of proposed scientific solutions. Singerman and Useche (2019) proposed replacing the voluntary character of the area-wide pest management programme with a mandatory component along with a self-enforcing feature to guarantee participation as key policy changes to make the programme successful. This top-down regulation could then serve to generate a bottom-up collective action, provided growers were given appropriate incentives and support.

The growing biosecurity threat to the Southern African citrus production areas has prompted the development of HLB and ACP Action Plans in South Africa and regional efforts in East Africa should ideally be tied in to these response actions, from citrus farmers on the ground, right up to national legislation to enforce restrictions on plant movement and controls at provincial and country boundaries. In Southern African countries, early warning surveillance has been initiated in Eswatini, Zimbabwe, Namibia, Angola, Zambia and Mozambique, with no *C*Las or ACP detection in these countries so far (Gebeyehu *et al.,* 2020). In South Africa, a suite of biosecurity preparedness actions has been developed; these include capacity building, awareness raising, detection and monitoring surveys in commercial orchards, farms, non-residential areas and border posts/entry points, all informed by a dedicated HLB and ACP Action Plan adopted by an HLB steering committee.

**Table 3.** Recommendations on surveillance and management for African countries.

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| Chemical control | Many are recommended however the lower risk products may be unavailable or costly. | • Only use pesticides recommended by the government. Select lower risk pesticides or selective insecticides if available/affordable to protect natural enemies.<br>• Rotate insecticides with different modes of action and follow all safety and operating advice.<br>• Buy from registered dealers. Use proper PPE and observe the re-entry/pre-harvest intervals of the product.<br>• Check for leaves that look yellowed or mottled with green midribs, and take collective action to control the disease vectors, if the disease is present. | • Use proper PPE and observe the re-entry/pre-harvest intervals of the product.<br>• Spray regime should be informed by effective monitoring of the psyllid using yellow sticky traps and visual inspections, ideally coordinated across commercial orchards in the region, to determine critical time for simultaneous pesticide application and prevention of spread.<br>• Edges and blocks on the farm periphery should be heavily monitored and prioritized for treatment.<br>• Elimination of inoculum sources should also be conducted outside commercial orchards, in | • Publish and make public the list of recommended chemicals for control of ACP which have low impact on beneficials.<br>• Sensitize regulatory authorities for initiating necessary actions aimed at fast-tracking the process of testing, validating and registering products for control with new active ingredients and adjuvants which may provide more effective and more environmentally-friendly ACP suppression.<br>• Preparedness for Areawide ACP Treatment Programme to allow for informed, coordinated insecticide treatment among |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | • Applications should be informed by monitoring and aim to minimize impact on natural enemies. Scouting and coordination amongst neighbouring smallholders is critical.<br>• Onset and duration of flushes should be monitored and young trees with multiple flushes prioritized for control.<br>• Soil drenches are best applied when the tree is flushing and during dry periods. Foliar sprays with different types of materials, including oil can be used during the rainy season.<br>• Broad spectrum foliar insecticides may be more suitable for mature trees (not when | rural and urban areas, and abandoned or poorly managed orchards to prevent secondary infection.<br>• Use pesticides recommended by government, especially those with low impact on beneficial organisms. Rotate insecticides with different modes of action or consider IGR products.<br>• Consider low-volume spraying to allow application over wider areas. | neighbouring orchards to maximise control, should the disease and vector be found more widely. |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | flowering) but control is challenged by asynchronous flushing and shorter residual effects. | | |
| Botanicals | Commercial neem products are available in some countries (e.g. Nimbecidine). Some essential oils tested in Africa (e.g. Ocimum sp.) | • Use recommended neem products, organic and "soft" foliar insecticides such as oils and soaps (horticultural spray oil, neem oil, kaolin clay, insecticidal soap) but applications may need to be made frequently when psyllids are observed (every 7 to 14 days) since these lack persistent effect.<br>• Interplanting with guava may help repel psyllids. Consider using homemade neem products to repel psyllids. | • Assess efficacy of natural products such as neem and interplanting with guava and adopt for use. | • Accept supporting data from other countries for registration of novel botanicals (e.g. anti-microbial peptides).<br>• Work with international agencies to test locally manufactured botanical pesticides, essential oils which maximise toxic effect or repellence of ACP/ACT and reduce side effects on beneficial arthropods. |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| Biological control | Native parasitoids present in South Africa for but hyperparasitoids can reduce effectiveness. | • Adapt specific cultural practices that conserve native natural enemies such as coccinellids and syrphids (e.g. use only low-risk, species-specific pest control products).<br>• Maintain crop diversity and flower strips comprising suitable wild plants to allow natural enemies to persist in the environment when psyllid numbers are low. | • Adapt cultural practices to augment naturally occurring arthropods (spiders, ladybirds, syrphids) to help suppress populations of psyllids. | • Work with international agencies to test candidate biological control agents for ACP following the IPPC guidelines.<br>• Explore the possibility of classical biological control through the introduction of specific parasitoids from Asia/countries where previously implemented (e.g. La Réunion). |
| Host plant resistance | No resistant citrus seedling trees or scion-rootstock combinations have as yet been identified. | • Choose varieties which are naturally more tolerant e.g. Tangelo nova, Tangerine Dancy and Tangerine Sugar Bell. | • Choose varieties which are naturally more tolerant e.g. Tangelo nova, Tangerine Dancy and Tangerine Sugar Bell. | • Facilitate multiplication of any current varieties showing more tolerance to HLB.<br>• Maintain international dialogue to help incorporate latest research into breeding programmes and new tools emerging from biotechnology, |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | | | molecular genetics and genomics research. |
| IPM | Many options but may be ineffective when just one method used, especially if psyllid becomes more ubiquitous. Need for greater grower information resources and sensitization to merits of IPM in citrus, for all pests and diseases. | • Check for the presence of psyllids in young flushes and carry out effective monitoring (bi-weekly) using branch tap method and/or sticky traps to inform management options. Use non-chemical methods wherever possible.<br>• Trees should be given adequate irrigation and nutrients to maintain optimal health and productivity but management practices which promote new flush such as hedging and topping and fertilization should be used judiciously. | • Check for the presence of psyllids in young flushes and carry out effective monitoring (bi-weekly) using tap method and/or sticky traps to inform management options.<br>• Maintain good records of agronomy, monitoring, interventions, yield etc and review regularly.<br>• Use scion-rootstock variety combinations with higher productive efficiency.<br>• Provide adequate fertilization and irrigation, higher planting density. | • Implementation of National Action Plan to include Area Wide Integrated Management Strategies to eliminate/contain the disease and mitigate further spread.<br>• Launch awareness campaign as part of a Regional Information Communication Strategy to highlight HLB threat (including grower/exporter information package, training materials on alert early warning, symptoms, seasonality and management).<br>• Facilitate cooperative approach for integrated management across the region (through |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | • Infected trees should be removed and replaced with disease free stock.<br>• Remove abandoned and unproductive citrus trees that may act as refugia.<br>• Use live windbreaks on the borders of orchards (>9ft tall) and keep trees relatively short for better visual inspection. | | stakeholder workshops, capacity building for detection and diagnosis, subsidized training in management and surveillance networks etc), including more focused, rapid action plans for hotspot areas. |
| Surveillance/ monitoring | | • Tree taps method used to monitor adults as well as sticky traps placed in the northern, southern and middle rows of citrus orchards and increased scouting of edges. | • Regular scouting of border trees is recommended: every 2–4 weeks, depending on the season and age of the tree.<br>• Scout young trees as often as once a week, mature trees once a month. Every 2 weeks is suggested as the normal protocol during periods of flushing. | • Mobilize resources and/or develop capacity for information network to communicate and share early warning systems, contingency plans, as well as high-quality monitoring data from known geographic locations/ incursion detection surveys with rapid sample analysis and reporting. |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | | • One central row should also be sampled, to determine if psyllid movement farther into the orchard has occurred. Sample a single flush from each of 10 trees along each border.<br>• Treatment is recommended before psyllids reach 0.5 nymphs per flush, or 2 of 10 flushes are infested. | • Quarantine and official orders for border control of plant/fruit movement.<br>• Review early detection tools such as canine-based detection, altered metabolic, volatile organic compounds for potential to support PCR based HLB diagnosis. |
| Good Agricultural Practice | No citrus certification scheme in Kenya and Tanzania. Lack of knowledge regarding HLB and vectors, poor grove management, local trade in infected material. | • Use disease-free planting material from certified sources.<br>• Proactive scouting for the vectors and rapid and safe removal /disposal of infected trees with symptoms of greening. | • Ensure citrus material has phytosanitary accreditation and track and trace system for provenance and distribution.<br>• Active and regular scouting and management to mitigate introduction of diseased material and subsequent spread. | National regulation and compliance scheme for citrus nurseries and suppliers to ensure certification and traceability scheme for clean planting material and appropriate quarantine measures to mitigate introduction and spread. |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | | • Keeping trees short for ease of monitoring and management.<br>• Good horticultural practices involving the application of optimal nutrition and irrigation to reduce tree stress and vulnerability. | • Establishment of screen houses and insect-proof nurseries/covers for susceptible young plantings and wind barriers at orchard edges.<br>• Training of nursery operators and technicians in all aspects of management, sanitation and movement protocols.<br>• Removal (rogueing) of infected trees. | |
| Tree pruning | Pruning of infected parts of the plant is a common practice in many parts of East Africa. The severe pruning results in maximum stimulation and the strongest regrowth. Severe root dieback occurs with HLB, and it is not known whether an affected tree can withstand the severe pruning to re-establish a canopy and produce an | • There is a rapid regrowth response of the pruned trees which may indicate that a pruning approach could be effective at rejuvenating the HLB-affected trees, and an alternative to tree removal and replanting in the short term but | • Fruit yields from pruned trees never surpass the yields from non-pruned trees possibly due to the severe pruning treatment.<br>• The effectiveness could be combined with enhanced foliar nutrition treatments, although | |

| Method | Availability in Africa | Recommendations for smallholders | Recommendations for commercial farms | Recommendations for government |
|---|---|---|---|---|
| | acceptable fruit yield. Severe pruning is unlikely to be cost effective in the longer term. | new flushes induced by pruning are attractive to psyllids so only light pruning is recommended in areas where disease is not endemic | the benefits only accrue in the early years. | |

# 4 Advice, information and communication

## 4.1 Sources of information on HLB control

Huanglongbing is still a new disease in Kenya, and consequently not much information is being disseminated on the proper identification of the disease and how to differentiate it from ACGD, as well as its vector. ICIPE has recently completed a project of citrus pests and diseases and one of the next steps suggested was to compile and document all the available information on the efficacy of different management options of HLB and its vector, learning from experiences in the USA, China and South Africa. Consequently, this would mean creating awareness among the farming communities, extension and quarantine personnel about this new disease and its vector (through mass media, ICT, training and communication materials in local languages). In terms of control, HLB does not lend itself to some of the common methods employed for most pests as the disease is often detected late when the citrus tree starts showing symptoms. Therefore, any recommendations communicated to the farmer should be efficacious, safe, sustainable, practical, available and affordable.

## 4.2 Criteria for control advice

We recommend the following criteria for control advice:

- Efficacy This is often assumed to be the most important criterion, even if this is not stated explicitly. If a practice is to be recommended there should be some evidence that it will be effective in at least some situations. Where a product has to be registered, this generally includes demonstration of efficacy, but many IPM practices do not involve a registered product. Results from controlled trials in an appropriate context are desirable, though not always available.

- Safety Even registered products can be hazardous to human health without precautions. Safety should thus be considered based on a consideration of how the product is likely to be used rather than whether recommended safety precautions are adequate. Some practices not requiring registration can also be hazardous, such as some plant extracts.

- Sustainability Possible effects on non-target organisms, such as pollinators, natural enemies and other organisms, should be considered. A control method may also have potential to create new problems, such as resurgence of other pests or pesticide resistance.

- Practicality Some methods may be impractical for some farmers, particularly those requiring elaborate safety precautions. Others may be only practical at a small scale.

- Availability Availability of regulated products is initially determined by their registration status, but even registered products may not be widely stocked if distribution is expensive and/or the perceived market is small. Unregulated inputs for some control methods may not be easily available, such as seeds of companion plants.

- Cost-effectiveness At the simplest level the cost of control must be less than the value of crop loss avoided, for it to be worthwhile. Opportunity and other costs may need to be considered.

## 4.3 Information resources and tools

Citrus Pest & Disease Prevention Program (CPDPP): Best practices in response to huanglongbing in California citrus. https://citrusinsider.org/wp-content/uploads/2019/06/Voluntary-Actions-Best-Practices-COMPLETE-FINAL.pdf (accessed 21 July 2020)

California Department of Food & Agriculture Action Plan for Asian Citrus Psyllid and Huanglongbing (Citrus Greening) in California. https://www.cdfa.ca.gov/citruscommittee/docs/ActionPlan.pdf (accessed 2 March 2021)

Animal and Plant Health Inspection Service, US Department of Agriculture: Citrus greening programme information including links to multi-agency response measures. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus/citrus-greening (accessed 2 March 2021)

Summary action plan and procedure for early detection and response to HLB and the Asian citrus psyllid entering South Africa. http://sana.co.za/wp-content/uploads/2013/01/DAFF-citrus-greening-action-plan-1.pdf (accessed 21 July 2020)

Invasive Species Compendium: This is a free encyclopaedic resource that brings together a wide range of different types of science-based information to support decision making in invasive species management worldwide. www.cabi.org/isc

Crop Protection Compendium: This is an encyclopaedic resource that brings together a wide range of different types of science-based information on all aspects of crop protection. It comprises detailed datasheets on pests, diseases, weeds, host crops and natural enemies. https://www.cabi.org/cpc

# 5 Recommendations

HLB presents a serious and rapidly-growing threat to citrus-growing regions across the whole of Africa. A successful response will require acceptance of the urgency of the situation, willingness to cooperate and persevere with management plans in the near- and mid-term, but above all, it will need an unprecedented degree of cooperation across many sectors. As demonstrated around the world, only through a coordinated effort by government officials, regulatory agencies, plant protection agencies, citrus growers, research institutions, extension workers and society can the wider introduction and spread of HLB be prevented and the vector contained to protect this valuable crop in the East African Region.

### High-level policy makers:

- Recognize the magnitude of the HLB threat (present and potential) and make policy decisions, backed by available economic estimates and science-based evidence
- Lobby for budgetary allocation to facilitate an immediate, official national response backed by policy and legislation, to enable coordinated (potentially mandatory) contingency action to curb the spread of the disease at the earliest opportunity
- Aim to create preparedness action plans across the East African Community and harmonize with established steering committees across the continent (e.g. South Africa's Emergency Plant Pest

Response strategy and HLB action plan) to mitigate and contain the disease. Consider use of incentives for replanting certified disease-free material, including HLB Safe Citrus Tree Production Systems

- Provide support for capacity building and knowledge exchange/dissemination across the entire citrus value chain to include early warning surveillance (track and trace), awareness raising, development of standard monitoring and diagnostic protocols and integrated management

**Regulators:**

- Strengthen the letter and enforcement of phytosanitary regulations on movement of citrus plants and alternate hosts
- Develop an HLB alert brochure followed by a comprehensive brochure on all aspects of management
- Regulate and implement protection of nursery stock in ACP-proof structures
- Plan and implement inspection of commercial and garden citrus and alternate hosts of ACP and HLB
- Prepare to impose strict quarantine and containment measures in hotspots, at country boundaries and at provincial boundaries with only nurseries certificated HLB-free permitted to move citrus trees
- Encourage track and trace system for movement control
- Fast-track the process of testing, validating and registration of products for the control of the HLB vector

**Researchers:**

- Undertake coordinated monitoring at the regional scale to create a dynamic picture of ACP demography, density and impact across the citrus-growing areas of Africa and share valuable data for vector management plans
- Carry out surveys to identify local natural enemies that can be used in augmentative and conservation biocontrol
- Test locally-available biopesticides and essentials oils and produce formulations that can maximise the toxic effect on ACP and reduce side effects on beneficials
- Timely and systematic communication of research outcomes and integration into national action plans
- Establish and support international research networking links to stay abreast of HLB research progress

**Advisory services:**

- Create a communications plan to raise awareness within the citrus production community/value chain, and public at large regarding dangers and risks associated with ACP and HLB
- Expand extension efforts to facilitate and coordinate judicious control of the psyllid vector if detected, promoting IPM and low-risk options for management, as well as rapid removal of infected trees in commercial, smallholder and abandoned citrus groves
- Creation of Citrus Health Management Areas to promote coordinated and integrated management

- Develop an array of cost-effective, clear and harmonized monitoring and impact assessment protocols to be followed for HLB studies

**Smallholder farmers:**

- Engage in good agricultural practice to maximise tree health, including good irrigation and fertilization (e.g. mulching, use of organic manure)
- Practice good preventative action by selecting healthy budwood and protect seedlings and flushing plants as a priority, with additional focus on grove edges
- Engage in early warning surveillance, with ACP scouting and trapping as a routine
- Treatment and removal of infected trees as a priority and replacement with healthy trees, with added protection from psyllid ingress in hotspot areas

**Commercial farmers:**

- Citrus nurseries are encouraged to proceed with changing over to citrus tree production in insect-secure structures
- Commercial growers need compliance agreements to ensure production of certified disease-free material and implementation of phytosanitary protocols regarding movement and elimination of inoculum
- Commercial nurseries should work towards an effective disease/vector management contingency programme
- Commercial farmers should engage in training on HLB/ACP scouting, diagnosis, monitoring and contingency management

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