HOLISTIC AREA-WIDE APPROACH FOR SUCCESSFULLY MANAGING CITRUS GREENING (HUANGLONGBING) IN MEXICO

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SUMMARY

The General Directorate of Plant Health (DGSV in Spanish) is recognized as the National Plant Protection Organization of the Federal Government of Mexico that acts under the Plant Health Federal Law. Some relevant plant protection programmes that Mexico is implementing include: The Huanglongbing (HLB) - Asian Citrus Psyllid (ACP) Programme, Mediterranean Fruit Fly Programme, National Fruit Fly Campaign, Pink Hibiscus Mealybug Programme and a permanent Phytosanitary Epidemiological Surveillance Programme to prevent the introduction and spread of regulated non-native pests. HLB or citrus greening is caused by the bacterium, *Candidatus Liberibacter* spp., and considered the most devastating citrus disease in the world. Once infected, it causes the death of orange, mandarin, grapefruit and lemon trees within 3 to 8 years. HLB is transmitted by the ACP, (*Diaphorina citri* Kuwayama), an insect vector widely distributed in most citrus producing regions of the world, including the citrus areas of Mexico. Until 2004, the disease only existed in Asia and Africa. It was first reported to occur in the Americas in 2004 (São Paulo, Brazil) and 2005 (Florida USA). In 2009, it was detected for the first time in Yucatán, Mexico. During that year, the national Mexican citrus production was 6.82 million tons (SIAP 2017). The economic impact evaluation by Salcedo-Baca et al. (2010) indicated that without the intervention of the Federal Government, HLB would be responsible for a reduction of the Mexican citrus production by 2.7 million tons in five years (39.6%). In spite of the spread of HLB, the citrus production in Mexico increased 11% to 7.56 million tons in 2015 (SIAP 2017). Today Mexico has 573 406 hectares (ha) of citrus compared to 545 947 in 2009, an increase of 5%. The first phytosanitary actions implemented on an area-wide basis were: (1) timely detection of HLB in agricultural and urban areas; (2) systematic elimination of infected trees in areas under surveillance; (3) control of the *D. citri* vector and (4) protection of propagative material in nurseries to avoid its infection. As a result of the successful HLB Programme implemented since 2008, adverse effects of the disease have largely been avoided. Management of HLB is organized through Regional Areas of Control (*Áreas Regionales de Control* or ARCOs), which implement the following area-wide measures: epidemiological surveillance and monitoring of psyllids based on criteria associated to climate and host presence in urban

and cultivated areas, and chemical and biological controls. From 2010 to 2015, 31 million parasitoid wasps *Tamarixia radiata* (Waterston) were produced and released in commercial citrus and backyard host areas of Yucatán, Quintana Roo, Campeche, Tabasco, Chiapas, Oaxaca, Hidalgo and Guerrero. The ARCOs are public-private organizations jointly operated by federal and state governments together with citrus grower associations. In 2016, the Mexican government allocated almost USD 8.5 million to the HLB Programme. With these actions, Mexico has largely mitigated the adverse effects of the disease while at the same time slightly increased citrus production. In addition, research programmes have been established together with scientific institutions to generate vegetative material with tolerance or resistance to the disease. Although the government has successfully implemented area-wide strategies for regional control, it is necessary to develop new and improved technologies to eliminate the vector, following the example of the Mediterranean fruit fly Programme in Mexico.

Key Words: Asian citrus psyllid, *Diaphorina citri*, *Candidatus Liberibacter*, economic impact, Mexican states, citrus production, *Tamarixia radiata*

1. INTRODUCTION

Huanglongbing (HLB) or citrus greening is a disease native to China and is considered one of the most destructive citrus diseases in Asian countries where its occurrence was originally reported more than a century ago (Bové 2006). More recently this bacterium reached the American continent together with its insect vector, the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama), capable of infecting and causing considerable damage to plants in the family Rutaceae (Alemán et al. 2007; Bové 2012).

The current geographic distribution of HLB extends to 12 countries in Asia, several islands in the Indian Ocean, Iran, portions of Africa, the Arabian Peninsula, Argentina, Brazil, Paraguay, Central America (except Panama), Barbados, Belize, Cuba, Dominican Republic, Guadeloupe, Jamaica, Martinique, USA (including Puerto Rico and the U.S. Virgin Islands) and Mexico (da Graça and Korsten 2004; Halbert and Manjunath 2004; NAPPO 2005; Bové 2006; Manjunath et al. 2008; Collazo et al. 2009; Trujillo-Arriaga 2011). In 2009, HLB was detected in Mexico for the first time in the state of Yucatán, and now is known to occur in 24 Mexican states (SENASICA 2017a).

The HLB-causing bacterium mainly attacks sweet orange and mandarins (da Graça and Korsten 2004) although all citrus varieties have shown varying degrees of susceptibility to infection as well as other members of the Rutaceae family. Currently, there is no successful control method to cure this disease, and as a result, infected trees die in the course of a few years.

This disease is caused by Gram-negative bacteria of the genus *Candidatus Liberibacter* (Bové 2006). There are three species of HLB-associated bacteria: *Ca. L. asiaticus*, *Ca. L. africanus* and *Ca. L. americanus*. All three have been described as the cause of HLB in different countries and climates world-wide (da Graça and Korsten 2004; Halbert and Manjunath 2004; Bové 2006; Wang et al. 2009), along with its insect vectors: ACP and the African psyllid, *Trioza erytreae* (Del Guercio). The first is the vector of *Ca. L. asiaticus* and *Ca. L. americanus*; while *T. erytreae* is the vector of *Ca. L. africanus*. A fourth species, *Ca. L. caribbeanus*, recently was identified from samples of ACP and *Citrus sinensis* (L.) Osbeck in Córdoba, Colombia. Efforts are underway to determine its pathogenicity and if it causes HLB symptoms (Keremane et al 2015).

The main symptoms of HLB are asymmetrical blotchy mottle yellowing in leaves, chlorosis, fruit drop and foliar loss and tree death. In addition to reducing the size and quality of fruit, the disease causes malformations and bad taste of fruit (Schwarz et al. 1973; Bové 2006). Although infected trees can remain productive for 5 to 8 years, the fruit are of poor quality (Halbert and Manjunath 2004).

To date, no control strategy is available that allows the immediate elimination of the pathogen, so its management in commercial citrus production areas is limited to the control of psyllid vectors through the application of insecticides, elimination of symptomatic trees with the aim of reducing levels of inoculum, isolation of affected areas by quarantine enforcement, and certification of pathogen-free propagative material (da Graça and Korsten 2004; Bové 2006; Manjunath et al. 2008; Gottwald et al. 2012). Because infected orchards become economically non-viable after 7 to 10 years, efforts focus on elimination of infected trees that have caused losses worth billions of dollars on a global basis (Gottwald et al. 1991; Vojnov et al. 2010).

In Florida, introduction of HLB resulted in major changes to pest management practices and corresponding costs. According to Singerman and Burani-Arouca (2017), average annual pest control consisted of two sprays for processed juice fruit and six sprays for fresh market grapefruit mostly to control several minor diseases, mites and weevils. Following a series of hurricanes in 2004 and 2005, that resulted in the catastrophic spread of citrus canker and forced abandonment of eradication actions, the number of sprays increased to 3–4 for processed juice oranges and 10 for fresh market grapefruit. After the discovery of HLB in August 2005 and citrus black spot in 2010, the number of treatments rose to 8–9 sprays for processed juice fruit and 14 sprays for fresh market grapefruit aimed at both disease and ACP control combined with additional fertilizer treatments.

Costs per acre of foliar sprays for producing processed oranges in south-western Florida rose from USD 185.63 in 2003/2004 to USD 666.00 (+240%) in 2014/2015 while fertilizer treatments went from USD 207.69 to USD 486.96 (+134%) during the same timeframes. By comparison, costs per acre of foliar sprays for fresh market grapefruit in the Indian River area increased from USD 493.08 in 2003/2004 to USD 1300.40 (+164%) in 2014/2015 while fertilizer treatments rose from USD 190.56 per acre in 2003/2004 to USD 452.55 (+137%) per acre in 2014/2015. These totals do not include other cultural control costs nor cost for tree replacement. It was found that area-wide control of ACP through Citrus Health Management Areas provides an estimated differential gross economic benefit of USD 714 (USD 1218) for 2012/2013 (2013/2014) (Singerman and Page 2016).

Over the period from 2012/2013 to 2015/2016, HLB caused a cumulative loss of USD 1672 million in grower revenues (average of USD 418 million annually) resulting in average annual economic impacts to the Florida economy of -7945 jobs, -USD 658 million in value added, and USD 1098 million in industry output (Court et al. 2017). Citrus bearing acreage in Florida diminished from 576 400 acres in 2005/2006 to 410 700 (-29%) in 2016/2017 while production value dropped from USD 1 491 136 in 2005/2006 to USD 1 032 227 (-30%) in 2016/2017 (USDA-NASS 2006, 2017).

2. THE HLB IN MEXICO

Mexico is the fourth largest producer of citrus in the world, with a total of 572 000 ha in production, yielding 7.8 million tons annually (Table 1).

Table 1. Areas under citrus production for Mexican states - 2015 (SIAP 2017)

State	Area (ha)	%
Veracruz	246 750	43.13
Michoacán	50 276	8.79
Tamaulipas	44 432	7.77
San Luis Potosí	37 505	6.56
Puebla	32 067	5.61
Nuevo León	31 789	5.56
Oaxaca	25 469	4.45
Colima	19 748	3.45
Yucatán	18 189	3.18
Tabasco	15 532	2.72
Sonora	8 523	1.49
Guerrero	7 135	1.25
Jalisco	6 841	1.20
Hidalgo	5 680	0.99
Campeche	4 731	0.83
Chiapas	4 725	0.83
Quintana Roo	3 089	0.54
Baja California Sur	2 870	0.50
Sinaloa	2 729	0.48
Nayarit	2 469	0.43
Morelos	610	0.11
Baja California	383	0.07
Querétaro	253	0.04
Zacatecas	246	0.04
Total	572 051	100.00

The major commercial citrus varieties grown in Mexico are orange (*Citrus sinensis*), Key lime (*Citrus aurantifolia*), Persian lime (*Citrus latifolia*), mandarin (*Citrus reticulata*) and grapefruit (*Citrus paradisi*) (Table 2). The value of this crop was estimate at USD 862 million (SIAP 2017).

HLB was first reported in samples of psyllids in July 2009 in Yucatán. In subsequent years more detections occurred in other states of the country (Table 3) (Trujillo-Arriaga 2014; SENASICA 2017a; SENASICA 2017b; SENASICA 2017c). In addition, infected psyllids were detected in Coahuila and Tamaulipas, where mechanisms of control and eradication of the disease vector have been implemented.

Table 2. Production and value of principal citrus varieties in Mexico - 2015 (SIAP 2017)

Citrus crop	Production (million tons)	Value (USD million)
Limes (Key & Persian)	2.33	461
Orange	4.52	345
Grapefruit	0.42	33
Mandarin	0.29	23
Total	7.56	862

Table 3. HLB detections in Mexico, after initial detection in the state of Yucatán in the month of July 2009 (Trujillo-Arriaga 2014; SENASICA 2017a; SENASICA 2017b; SENASICA 2017c)

State	Detections of HLB	State	Detections of HLB
Quintana Roo	August, 2009	Tabasco	December, 2012
Jalisco	December, 2009	Guerrero	March, 2013
Nayarit	December, 2009	Puebla	September, 2013
Campeche	March, 2010	Zacatecas	September, 2013
Colima	April, 2010	Coahuila	December 2013*
Veracruz	June, 2010	Oaxaca	April, 2014
Sinaloa	July 2010*	Tamaulipas	June 2014*
Michoacán	December, 2010	Querétaro	October, 2015
Morelos	December 2010*	San Luis Potosí	October, 2015
Chiapas	March, 2011	Nuevo León	December, 2015
Sonora	April 2011*	Veracruz	December, 2015
Hidalgo	April, 2011	Tamaulipas	December, 2015
Baja California Sur	April, 2011	Baja California	January, 2016
Nuevo León	August 2011*	Morelos	August, 2016
San Luis Potosí	September 2011*	Sonora	March, 2017

* Infective psyllids

As of September 2017, HLB is recorded to infect plant material in all 24 citrus-producing states of Mexico (Fig. 1), where it has been detected in a total of 450 municipalities (SENASICA 2017a).

In Persian lime production in Yucatán, the presence of *Ca. L. asiaticus* caused a reduction in weight of the fruit (17.3%) and a decrease in the volume of juice (18.6%) (Flores-Sánchez et al. 2015). In Key lime, experts estimated a reduction of 183 168 tons should HLB become established throughout Mexico (Salcedo-Baca et al. 2010).

3. RESPONSE TO HLB IN MEXICO

The Mexican citrus industry, federal and state plant protection agencies responded to this situation with the following area-wide phytosanitary actions to manage the HLB disease: epidemiological surveillance in commercial orchards, urban areas, and sentinel gardens; and, chemical and biological controls of the insect vector in both backyards and commercial orchards in those regions where the weather conditions favour infection.

Management of HLB is organized through Regional Control Areas (ARCOs), which implement the area-wide measures, and allow for coordination of monitoring, biological and chemical control actions across hundreds of ha; thereby preventing outbreaks from expanding to thousands of ha (SENASICA 2012).

Figure 1. Areas in 24 states of Mexico with presence of HLB as of September 2017 (source SIRVEF 2019).

Since 2002, the vector of HLB has been present in Mexico, posing a significant risk for spreading HLB disease. Following the detection of HLB in Belize during 2008, the National Health, Food Safety and Food Quality Service (SENASICA in Spanish), of the General Directorate of Plant Health (DGSV in Spanish), initiated in 2008 a national priority campaign in 24 citrus states of the country. The aim of the programme was to detect the disease in a timely fashion and provide protection to more than 570 000 ha of citrus. A series of protocols were developed and revised by DGSV with input from State Plant Health Committees and the affected industry (SENASICA 2008a, 2009).

In 2009, the programme received an allocation of USD 2.56 million that allowed for monitoring the disease in 77 192 ha of commercial citrus and backyard trees (Sanchez 2013). As a result of these actions, the first detection of HLB found in infected ACP occurred in July 2009 in the town of Cuyo in the municipality of Tizimín, Yucatán (Trujillo-Arriaga 2011). In that same year and the following years, there were more detections in other states of the country (Table 3).

Due to the importance of the HLB to the Mexican citrus industry, efforts also were made at the federal and scientific levels to involve national universities and research centres to develop better management strategies, as well as better methods for the timely phytosanitary surveillance of the disease and the insect vector. To this end, SENASICA-DGSV established the legal bases for action as follows:

3.1. *Phytosanitary Regulation*

With the detection in 2009 of HLB in Tizimín, Yucatán, the federal government issued as a matter of emergency, the Mexican Official Emergency Norm NOM-EM-047-FITO-2009, which outlined and established phytosanitary actions to mitigate the risks of introduction and dispersal of HLB in Mexico (DOF 2009). It outlined the phytosanitary actions to implement a monitoring programme that included sampling, diagnosis, inspection and surveillance to assess any new introduction and further spread of HLB in the country and, where appropriate, the application of phytosanitary measures for its management. These included the delimitation of infected areas under phytosanitary control, the removal of infected material, the application of vector control methods, sampling, inspections, and restriction of the movement of vegetative material. The same actions are established in the "*Protocol of Action to the Emergency by the Detection of HLB*" and are supported under the agreement that discloses the phytosanitary measures which should be applied for the control of HLB and its vector (DOF 2010). In addition, the DGSV and the State or Regional Technical Working Group issued the Protocol to establish the ARCOs (SENASICA 2012) for HLB and ACP.

3.2. *Sampling and Monitoring to Assess HLB Distribution*

With the presence of HLB in Mexico, one of the main activities carried out in all citrus-producing states of the country was an assessment of the presence of the disease through the collection of plant material and adult *D. citri*. Priority was given to the commercial areas of Key lime, Persian lime and orange. When symptomatic plant material was found, photographs were taken and sent through the Digital Diagnostic System (SIDIADI), so the trained technical staff, based on the visual symptoms, determined whether the material was suspect of HLB infection and further sampling was required. In commercial orchards, sampling of *D. citri* was done mainly in trees located along the periphery of the orchards, where 1 to 100 adults were collected and analysed by molecular techniques for presence of HLB. Urban areas (parks, tree-lined boulevards, harbours, etc.) were also sampled.

3.3. *Phytosanitary Diagnosis*

Given the need for timely diagnoses, four official laboratories were used where plant material and psyllids could be examined: 1) the National Phytosanitary Reference Center (CNRF) in Tecámac, Mexico state, 2) the National Quarantine Station of Epidemiology and Plant Sanitation in Querétaro; 3) State Committee of Plant Protection of Yucatán, and 4) State Committee of Plant Health of Colima. In addition, eight private laboratories were approved to assist with these diagnoses. Furthermore, the DGSV has a mobile phytosanitary diagnostic unit with adequate equipment required for HLB *in situ* detections such as bioclimatic chambers, real-time PCR and End-Point PCR. This excellent infrastructure reduced the response time to a minimum and allowed speedy decision-making to apply local or regional phytosanitary strategies as described in 3.4 through 3.6 below.

3.4. Cultural Control

After an initial find of HLB positive vegetal material in a new area, the cultural control consisted of removing infected plants to avoid further spread from or possible resurgence of infection from new outbreaks. The following phytosanitary measures were implemented:

- All trees with symptoms of HLB should be eliminated within 5 days. For detections in commercial orchards, the owners were responsible for surveying all trees within their groves for the disease. Plants with symptoms were marked with a plastic tape indicating the branch(es) exhibiting symptoms. Plants located on the outer rows of orchards where the symptomatic plants were detected, were also marked with tape. Technical personnel checked each suspect orchard and confirmed whether or not the symptoms were caused by HLB. All positive cases were georeferenced. It was not considered necessary to sample these plants and technical personnel immediately removed the plants based upon a visual diagnosis. After tree removal, herbicides were applied to the stump. It is important to note that pruning cannot be used to manage HLB control and that replanting is not recommended as new plants are more susceptible to the disease (SENASICA 2010a; DOF 2010).
- In 2010 the Mexican government performed these activities in the following states with presence of HLB: Campeche, Colima, Jalisco, Michoacán, Nayarit, Quintana Roo, Sinaloa, and Yucatán. In these entities the exploration was carried out in 1553 localities, in which 1 127 275 citrus plants and 306 138 plants of lakeview jasmine (*Murraya paniculata* (L.) Jack) were inspected in search of suspicious symptoms and psyllids carrying the bacteria. Likewise, 17 539 citrus trees in orchards and 77 522 of lakeview jasmine in backyards were eliminated, as well as 5037 trees of Mexican lemon, Persian lemon and orange, in commercial orchards and 1 360 626 nursery plants (SAGARPA 2011).
- The presence of HLB in all the plants was determined by searching for symptoms and sending samples to the laboratory for diagnosis by molecular techniques, including those in the proximity of citrus nurseries. All hosts present in nurseries without anti-aphid mesh protection were removed. When a detection occurred in a backyard, it was the responsibility of trained Auxiliary Plant Protection Organization staff to identify HLB symptoms (SENASICA 2010a; DOF 2010).
- In orchards where at least 28% of plants showed HLB symptoms and where the clinical diagnosis was positive for the disease, all trees in the orchard were removed within a period of no more than five calendar days. The review of orchards in an outbreak area is being implemented permanently, with a review of such orchards done every 3 weeks to know the status of the disease (SENASICA 2010a; DOF 2010).

Currently HLB control is still based on eradicating sick trees in the states with new detections. In areas with high incidence of HLB disease, the growers have opted to implement intensive nutrition programmes to extend the productive life of the affected plantations. The strategies of the federal government campaign are directed towards the control of the ACP, through the establishment of the ARCOs, which implement the biological and chemical control activities in their respective regional areas (SENASICA 2017d).

3.5. *Biological Control of the Vector*

Augmentative biological control is a strategy that plays an important role in reducing the population density of *D. citri*, and its area-wide use in ARCOs has significantly contributed to reducing adverse environmental effects and minimizing interference with natural control of agricultural pests as a result of using agrochemicals (DGSV 2016).

In response to the detection of HLB and its vector *D. citri* in Mexico and to mitigate its threat for citriculture, SENASICA-DGSV and the CNRF established a *Biological Control Programme for Asian Citrus Psyllid*, as a complementary strategy to the integrated management of the HLB vector. The main activities in this programme have been:

- Search and selection of biological control agents of *D. citri*
- Mass-production of the species-specific ectoparasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae)
- The release of adult parasitoids in specific areas not subject to insecticide application (such as urban areas, inaccessible areas, organic orchards, abandoned vegetable gardens, backyard host trees, orchards adjacent to urban areas, areas under integrated pest management, and protected natural areas or reserves)
- Assessment of the effectiveness of biological control agents in the laboratory and in the field
- Training and public education on the recognition and use of biological control agents of ACP
- Advice on the design of rearing facilities for *T. radiata*
- Optimization of the mass-production process of the parasitoid
- Research on different strains of *T. radiata* present in Mexico and their regional impact
- Research on the use of strains of entomopathogenic fungi, and finally
- Obtaining national and international support on parasitoid rearing (FAO-SENASICA 2013).

Therefore, the DGSV through the CNRF, established collaborative agreements with the State Committees of Plant Protection in the states of Colima and Yucatán in 2009. The first agreement with the Entomophagous Insects Department of the National Reference Center for Biological Control (CNRCB), based in Tecomán, Colima, aims to generate technology (basic and applied) for the use of biological control agents of *D. citri*, as well as the production of *T. radiata* in Tecomán, Colima; while the second agreement with the Regional Mass-Rearing Laboratory of *Tamarixia radiata* in the Southeast has as its sole objective the mass-production of the parasitoid in Merida, Yucatán; both agreements are coordinated by the CNRCB (FAO-SENASICA 2013).

From 2010 to 2015, 31 million *T. radiata* were produced and released in citrus orchards and abandoned, urban and backyard areas of Yucatán, Quintana Roo, Campeche, Tabasco, Chiapas, Oaxaca, Hidalgo, and Guerrero. These release activities were supported by the State Plant Health Committees, who are responsible for transporting and release of parasitoids to the infested areas (CNRCB-CNRF-DGSV 2016). Many of these areas began with a parasitism rate ranging between 3-26% that increased to 70-85% after augmentative releases (SENASICA 2016).

The recommended release rate is 100 parasitoids every 50-100 linear meters depending on the level of ACP infestation and density of host plants. If on average more than 20 *D. citri* nymphs were observed per tree shoot, 100 parasitoids were released every 50 meters (SENASICA 2015). The releases are carried out with a minimum interval of one month and a maximum of 3 months (DGSV 2016). These releases directly and indirectly benefit hundreds of growers.

With respect to the use of entomopathogenic fungi as a complement to the control of *D. citri* populations, the following research activities were established in support of the Biological Control Programme of the ACP:

- Exploration of entomopathogenic fungi
- Selection of isolates of entomopathogenic fungi candidates for the control of immature and adult stages of *D. citri*
- Evaluation of conidia production
- Evaluation of types of entomopathogenic fungi formulation
- Evaluation of fungal formulations in the field
- Evaluation of application equipment; and
- Biosafety tests (FAO-SENASICA 2013).

By 2016 three strains of *Isaria javanica* (formerly *fumosorosea*), CHE-CNRCB 303, 305 and 307, formerly Pf15, Pf17 and Pf21, respectively, as well as one of *Metarhizium anisopliae* (CHE-CNRCB 224, formerly Ma59), had been identified. Laboratory tests achieved 93-100% mortality in nymphs and up to 95% in adults of *D. citri*, respectively. Applications of entomopathogenic fungi in preliminary field trials reduced psyllid populations from 48 to 90% (FAO-SENASICA 2013). In 2012 and 2013, the application of two strains of entomopathogenic fungi (Ma59 and Pf21) was carried out on 15 932 ha in the states of Colima, Hidalgo, Jalisco, Nayarit, San Luis Potosí and Veracruz (CNRCB-CNRF-DGSV 2016).

3.6. Chemical Control

In other countries, HLB disease has been managed mainly through the suppression of vector populations using synthetic insecticides. In Mexico, only the use of insecticides authorized by COFEPRIS (DOF 2010) are recommended in accordance with the use of products that have been approved and shown to be efficacious in other countries. Cortéz-Mondaca et al. (2010) conducted tests of the effectiveness of conventional synthetic and organic insecticides with different modes of action, including botanical extracts, mineral oils, soaps, entomopathogenic detergents and growth regulators. Based on these results, the National Campaign against HLB has been rotating the use of the following active ingredients according to the specific local situations: thiamethoxam, imidacloprid + beta cyfluthrin, mineral oil, bifenthrin, tricarboxyls, chlorpyrifos, imidacloprid, dimethoate, thiamethoxam + lambda cyhalothrin, bifenthrin + zeta cypermethrin, azadirachtin, bifenthrin + abamectin, detergent and lime oxide (SENASICA 2012).

In Mexico, chemical control is performed by the ARCOs. The application time is determined by the population dynamics of *D. citri* and the phenology of the citrus in each region. The spraying is done 2-3 times in all the orchards that are part of an ARCO within a two-week period of time. The State Plant Health Committees responsible for HLB disease management oversee the actions taken by ARCOs and inform growers about the timing of annual applications, and the overall pesticide management programme. ARCOs are responsible for any pesticide applications (DGSV 2016). In 2015 and 2016, 216 566 and 273 318 commercial ha of citrus were sprayed, respectively (SENASICA 2017b).

3.7. *Vegetative Material*

The Mexican Official Standard NOM-079-FITO-2002, “Phytosanitary Specifications for the Production and Mobilization of Propagation Material Free of Citrus Tristeza Virus and Other Pathogens Associated with Citrus”, and the agreement that discloses the phytosanitary measures to be applied for the control of HLB and its vector (DOF 2002, 2010; SENASICA 2010a) establishes requirements for propagation and certification of citrus nursery stock through Certified Production Units as a means of providing disease-free trees for commercial sale and planting purposes.

3.8. *Training and Outreach*

Two international workshops on ACP and Citrus Huanglongbing were held in Mexico during 2008 and 2010, respectively, with the objective of providing training on disease diagnostics to technical staff in charge of field monitoring and sampling. Experts were invited from infected countries such as Belize, Brazil, China, Cuba and the USA, to share their experiences on management and disease prevention practices (SENASICA 2008b, 2010b). In addition, three events on quarantine pests of citrus were organized in 2009, 2011, and 2013 (SENASICA 2013).

In order to create greater awareness among the general public about HLB and the risks posed, an additional 34 667 training events were organized between 2008 and 2011 in different regions of the country. Attendees were encouraged to participate under the motto "All against HLB of citrus and its vector".

In addition, billboards along roadways and avenues in rural villages and information disseminated using printed triptychs, posters, flyers, technical files, postcards, radio spots and videos were used to educate all involved. These materials invited the public to be on the alert for symptoms of HLB in their commercial and backyard orchards, and to immediately report HLB symptoms to the local plant health boards in their region (Trujillo-Arriaga 2011).

4. HLB IMPACT ON CITRUS PRODUCTION FROM 2009 TO 2015

In 2009, the year when HLB was first detected in Mexico, the area planted with lime, orange, mandarin and grapefruit comprised 523 321 ha yielding a total of 6.82 million tons of fruit (SIAP 2017). That same year, SENASICA and the Inter-American Institute for Cooperation on Agriculture (IICA) commissioned a study to estimate the expected impact of HLB in Mexico (Salcedo-Baca et al. 2010).

Various scenarios were developed to assess potential impacts to the industry and economy with and without any governmental intervention. Under the low impact scenario, the study estimated that within five years following the establishment of the disease a loss of 2.7 million tons of citrus fruit would occur nationwide with an overall reduction of 39.6% in orange, 33% in grapefruit, 17% in mandarin, and 10% in lime production respectively. Under the high impact scenario, losses would increase to 3 million tons of fruit equivalent to 41% of orange, 53% of grapefruit, 26% of mandarin, and 18% of lime production.

Figure 2. National citrus production in Mexico after HLB arrival in 2009 (SIAP 2017).

Six years after HLB was first detected in Mexico, however, with governmental intervention, the estimated losses in citrus production have yet to occur. Although citrus production in 2010 and 2012 decreased slightly, annual yields have remained fairly stable and have slightly increased by 0.7 million tons above the 2009 level (Fig. 2 and Table 4).

In 2016, the Mexican government allocated approximately USD 8.5 million to the HLB Programme. As a result of the successful application of the area-wide actions outlined above, Mexico largely mitigated the adverse effects of the disease, while at the same time slightly increasing citrus production. In addition, research programmes have been promoted among scientific institutions to generate propagative material tolerant or resistant to the disease.

Table 4. State-by-state comparison of citrus production in Mexico - 2009 and 2015 (SIAP 2017)

State	Production (tons) 2009				Production (tons) 2015			
	Orange	Lime	Mandarin	Grapefruit	Orange	Lime	Mandarin	Grapefruit
Aguascalientes	23	0	0	0	14	46	0	0
Baja California	3,499	2,978	192	96	3,260	1,171	0	183
Baja California Sur	29,303	144	0	66	49,995	309	22	495
Campeche	37,018	5,953	215	15,014	25,122	10,325	332	18,955
Chiapas	15,983	4,185	452	0	16,027	7416	404	0
Colima	3,977	423,040	0	0	4,496	191890	0	158
Durango	804	856	0	612	785	702	0	577
Guanajuato	0	3	0	0	0	12	0	0
Guerrero	4,189	78,404	230	66	4,435	71,867	138	9
Hidalgo	45,481	2,589	262	0	59,041	2,231	239	0
Jalisco	6,779	30,351	6	1,310	6,386	81,198	0	740
México	327	1,227	0	0	234	1,190	39	0
Michoacán	4,374	414,562	75	59,559	3,489	670,613	0	49,566
Morelos	4,430	3,660	82	160	4,857	4,438	190	144
Nayarit	1,576	14,093	41	5	712	18,423	19	0
Nuevo Leon	296,973	0	35,892	17,734	313,439	0	45,751	26,201
Oaxaca	60,626	176,182	0	5,440	56,290	245,137	0	1,161
Puebla	254,841	48,352	680	11,160	214,175	28,211	51,746	5,067
Querétaro	1,345	47	24	0	2,446	26	5	0
Quintana Roo	32,289	1,107	32	0	16,841	26,222	112	0
San Luis Potosi	431,567	8,599	20,358	55	337,717	21,986	22,212	62
Sinaloa	16,970	2,173	1,420	5,195	19,320	1,648	446	2,887
Sonora	167,371	988	581	17,500	142,445	1,564	3,059	16,689
Tabasco	81,519	80,939	380	850	81,451	83,141	387	845
Tamaulipas	539,526	46,411	54,235	29,083	668,935	121,200	11,618	46,544
Veracruz	2,058,040	514,728	102,046	256,064	2,336,427	659,034	147,345	248,927
Yucatán	94,534	104,777	6,441	11,704	147,107	74,463	6,885	5,106
Zacatecas	128	0	72	0	74	1,605	130	0
Total	4,193,484	1,966,345	223,718	431,671	4,515,520	2,326,068	291,078	424,315

Although the government has successfully implemented area-wide strategies for regional control, it is necessary to further develop new and improved technologies. Mexico, Belize and USA have formed a Tri-National Working Group for purposes of technical exchange, information sharing, planning, coordination, and identification of research priorities.

Mexico currently has 573 406 ha of citrus corresponding to 335 019 ha of oranges, 180 209 ha of limes (Key and Persian), 21 297 ha of mandarin, 17 590 ha of grapefruit, 12 736 ha of tangerine, 5238 has of tangelo and 1317 ha of sweet limes, with an estimated annual production of 8 million tons per year, and whose production value is approximately USD 20 424 million pesos (approximately USD 1.12 billion dollars) (SIAP 2017).

The first phytosanitary actions implemented on an area-wide basis were: timely detection of HLB in citrus and urban areas, elimination of infected plants in areas under control, suppression of the *D. citri* vector, and protection of propagative material within enclosed or screened nurseries.

5. CONCLUSION

In conclusion, as a result of the successful HLB Programme, citrus losses have been largely avoided in Mexico despite the fact that the disease has now been detected in all states of the country where commercial citrus is produced. Six years after the disease first appeared, the surface for the four principal citrus varieties actually increased by 5%, from 523 321 to 553 671 ha (Fig. 3) (SIAP 2017).

Figure 3. Expanding national areas of the four principal *Citrus* spp. produced in Mexico after the HLB arrival in 2009 (SIAP 2017).

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