Plant health and crop protection

Review article

Identification of technologies and methods for the early detection of Huanglongbing (HLB) through scientometrics in scientific articles and patents

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Abstract

The aim of this study was to identify scientific and technological trends in the detection of Huanglongbing (HLB) or "citrus greening", one of the most devastating diseases for citrus worldwide, as well as the main actions that are being carried out to mitigate the impact of the disease in the citrus-producing sector. The rapid and timely detection of this disease is key for producers because corrective actions can be proposed, which prevent a total loss of production, an effective cure is still unknown. A review of publications in scientific and technological databases was carried out to fill the gap knowledge, gathering data available between 2007 and 2018. The review was based on a technology surveillance methodology. The results were analyzed and processed, employing the Matheo Analyzer software, which identified topic variables for future studies. From the results obtained, it was evident that the United States and China are the countries that have a notable interest in the subject. In the area of early detection of HLB, the main research actors are the University of Florida and the USDA (United States Department of Agriculture). Among the early detection technologies and methods for this disease to be considered, several relevant areas of research and technological development were observed, including remote sensing from electromagnetic radiation captured by sensors. Another research area of interest was biochemistry and genetics, where molecular identification techniques, such as isothermal amplification and rapid polymerase chain reaction (PCR), are highlighted.

Keywords: *Candidatus* Liberibacter spp., citrus, *Diaphorina citri*, greening, technological surveillance

Identificación de tecnologías y métodos para la detección temprana del Huanglongbing (HLB) a través de cienciometría en artículos científicos y patentes

Resumen

El objetivo planteado para esta revisión de literatura científica y de patentes consiste en identificar las tendencias científicas y tecnológicas en la detección del Huanglongbing (HLB) o enverdecimiento de los cítricos, así como las principales acciones que se están adelantando para mitigar su impacto en la citricultura mundial. La detección rápida y oportuna de esta enfermedad es clave para los productores, debido a que se pueden plantear acciones correctivas que eviten una pérdida total de la producción, partiendo de que aún no se conoce una cura para la misma. Para lograr los resultados esperados, se realizó una revisión de las publicaciones en bases de datos científicas y tecnológicas, disponibles entre 2007 y 2018, a partir de una metodología de vigilancia tecnológica. Los resultados fueron analizados y procesados a través del *software* Matheo Analyzer, lo que permitió identificar las variables de futuro para el tema de trabajo. Se evidenció que Estados Unidos y China son los países que presentan un notable interés en el tema relacionado. Dentro de las instituciones líderes en investigación sobre detección temprana del HLB, se resaltan la Universidad de Florida y el Departamento de Agricultura de Estados Unidos (USDA, por sus siglas en inglés). Entre las tecnologías y métodos de detección temprana para esta enfermedad, se encontraron como áreas relevantes de investigación y desarrollo tecnológico la teledetección a partir de la radiación electromagnética captada por sensores, y el área de bioquímica y genética en la que se destacan las técnicas de identificación molecular como la amplificación isotérmica y la reacción en cadena de la polimerasa (PCR).

Palabras clave: *Candidatus* Liberibacter spp., citrus, *Diaphorina citri*, enverdecimiento, vigilancia tecnológica

Introduction

World citrus (*Citrus* spp.; Rutaceae family) production is estimated in more than 105 million tons per year, of which more than half corresponds to orange, which makes this fruit the number one crop produced worldwide (Cubero, Lee, Aleixos, Albert, & Blasco, 2016). Currently, this crop is seriously affected by the appearance of a bacterial disease that has generated huge economic losses in countries such as the United States, Mexico, and Brazil; it is called Huanglongbing (HLB) or yellow dragon disease, and is one of the most devastating diseases of citrus fruits (Badaracco, Redes, Preussler, & Agostini, 2017; Bové, 2006; Cubero et al., 2016), with losses of 3 billion dollars in annual production value reported in Florida (United States) alone (Sankaran, Maja, Buchanon, & Ehsani, 2013).

This bacterium is transmitted from one tree to another through insect vectors: *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in Asia and America, and *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) in Africa (Bové, 2006), as well as with plant propagation material such as seedlings, grafts, and buds (Garcés-Giraldo, 2012; León & Kondo, 2017; Manjunath, Halbert, Ramadugu, Webb, & Lee, 2008).

The disease was first detected in China in 1919 (Vojnov, Do Amaral, Dow, Castagnaro, & Marano, 2010); later, in 1928, citrus trees with similar symptoms were found, but this disease was not officially recognized until 1937 (Bové, 2006). HLB can affect almost all citrus cultivars, but *Citrus sinensis* (L.) Osbeck, tangelos (*Citrus* × *tangelo* J.W. Ingram & H.E. Moore), and mandarins (*Citrus reticulata* Blanco) are the most susceptible; on the other hand, *Citrus* × *aurantifolia* (Christm.) Swingle, the bitter orange *Citrus* × *aurantium* L., and *Citrus trifoliata* L. are the least susceptible (Knapp et al., 2004). According to Abdullah, Shokrollah, Sijam and Akmar (2009), only some cultivars of other species such as *Citrus indica* Tanaka and *Citrus macroptera* Montrouz have shown some tolerance or possible resistance to the bacterium. Kumquat *Fortunella margarita* (Lour.) Swingle, previously reported as resistant to HLB, was recently reported infected and mottled (Tsai, Hung, Su, & Liao, 2006).

Currently, 100 % effective control against the disease is not known, except preventing the trees from becoming infected (Bové, 2006), so identifying it as quickly as possible allows farmers to take the necessary measures for its management. This must be done immediately after the presence of the disease is confirmed; eradication is one of the main recommendations for its management (León & Kondo, 2017), or the use of treatment and control techniques such as those described by Al-Jumaili and Ehsani (2015).

The visual detection of this disease is difficult because its incubation period is long and can be influenced by the age and health of the tree, so there can be twice as many asymptomatic infections compared to the visually symptomatic ones (Gottwald, 2010). Due to this, it is necessary to identify scientific and technological solutions that allow producers to detect the early presence of the disease to mitigate the impact it could generate.

Given this problem, the search, treatment, and analysis of information processes from scientific and technological databases are a useful tool to track possible innovative solutions that support the decisions of producers. Furthermore, it must be understood that the number of published studies and granted patents are an important indicator to measure the development trend of a certain research area (Wang, Pan, Ke, Wang, & Wei, 2014).

Reviews carried out by other authors, such as Iftikhar, Rauf, Shahzad and Zahid (2016), have identified solutions for disease control, including phytosanitary techniques, control over vector populations, cultural practices, chemotherapy and the distribution of disease-free material, and has set aside all the rapid detection techniques. Other reviews carried out by Valdés et al. (2016) have focused on the promptness of the detection because the most widely used mechanism is the tree-to-tree inspection, which is costly and labor-intensive. Therefore, polymerase chain reaction techniques, as well as other image treatment techniques, stand out. It should be noted that none of these reviews shows a structured review model and treatment of scientific or technological database results that support its findings and allow evidencing the evolution of this topic over time.

In a knowledge-based economy, the ability to discover the innovation seeds in basic and applied research is the source of competitiveness, economic growth, and development of technological applications (Shibata, Kajikawa, & Sakata, 2011); therefore, scientific articles and patents are part of the research infrastructure involved in the construction of public knowledge (Hemmungs-Wirtén, 2015).

However, exponential growth in scientific and technological production is necessary to use technological methods and tools that allow the efficient treatment of these large volumes of data and convert them into strategic information. There are different technological tools for the treatment of data obtained from sources such as Scopus for scientific articles, and PatentScope for patents, including among others, Vosviewer, SciMAT, BibExcel, CiteScpace, and Matheo Analyzer. The latter was used in this review, since it is a payment software that allows analyzing large volumes of data, creating a control panel, refining the extraction, displaying the information graphically, and creating rules for the identification of information that other free software does not contemplate.

Huanglongbing citrus disease

In citrus fruits, the HLB symptoms appear on the leaves as yellowish spots with the appearance of asymmetric mottling and stunt growth in developing plants, as well as the production of small and deformed fruits with inverted coloration (figure 1). The fruits can fall off the tree prematurely (Bové, 2006; Gottwald, Graça, & Bassanezi, 2007). Leaf symptoms can be confused with nutritional deficiencies caused by pests and other diseases (Bové, 2006; Gottwald, Graça, & Bassanezi, 2007).

Figure 1. Symptoms of HLB in fruits (deformity) and leaves (mottling)
Source: Takumasa Kondo (AGROSAVIA)

Characterization of the disease cycle

Three forms or species of bacteria have been associated with the HLB disease: *Candidatus* Liberibacter africanus, *Candidatus* L. asiaticus, and *Candidatus* L. americanus, all present in Brazil (Laflèche & Bové, 1970). The disease infection cycle develops from the vector insect known as *Diaphorina citri*, which through feeding, can inoculate the bacteria in the plant phloem (Augier, Gastaminza, Lizondo, Argañaraz, & Willink, 2006).

In the American continent, *C.* Liberibacter asiaticus and *C.* Liberibacter americanus are found (Laflèche & Bové, 1970), so they are of interest in this research. Both generate a progressive obstruction of the phloem by an accumulation of starch (León & Kondo, 2017), hindering the normal transport of nutrients in the plant; this, in turn, generates damages in leaves and fruits, and finally, the productive and physiological death of the infected tree occurs (Bayer, 2015). The infected vector continues its feeding by migrating from tree to tree and leaves eggs that subsequently continue to spread the disease throughout the orchard (Bayer, 2015).

The *Diaphorina citri* Kuwayama vector (Asian citrus psyllid)

HLB or citrus greening is a bacterial disease caused by *C.* Liberibacter spp. and transmitted by two species of insects: *D. citri* and *T. erytreae* (Hall, 2008), of which only the first species is found in Colombia (León & Kondo, 2017), and it is associated with the transmission of *C.* L. asiaticus. The psyllid vector is distributed in tropical and subtropical regions. In South America, it has been reported in Argentina, Brazil, Colombia, Paraguay, Uruguay and Venezuela (Augier et al., 2006; Cermeli, Morales, Perozo, & Godoy, 2007; European and Mediterranean Plant Protection Organization [EPPO], 2006; Instituto Colombiano Agropecuario [ICA], 2010).

In Colombia, *D. citri* was first reported in 2007 in the department of Tolima. Subsequently, its presence was reported in the departments of Antioquia, Atlántico, Bolívar, Boyacá, Caldas, Casanare, Cauca, Cesar, Córdoba, Cundinamarca, Huila, La Guajira, Magdalena, Meta, Norte de Santander, Quindío, Risaralda, Santander, Sucre and Valle del Cauca (ICA, 2010; Kondo, Quintero, Campuzano, Wyckhuys, & Heraty, 2012).

Diaphorina citri has an extensive list of host plants, mainly belonging to the Rutaceae family (León & Kondo, 2017). Halbert and Manjunath (2004) recorded 25 genera of plants of the Rutaceae family as the main hosts of *D. citri*. In Colombia, myrtle or *murraya* (*Murraya paniculata* (L.) Jack) and swinglea (*Swinglea glutinosa* (Blanco) Merr.) stands out as the species that most favor the dispersion of *D. citri* (León & Kondo, 2017).

This insect goes through five nymphal stages, which vary in size after each molt; the last stage is characterized by presenting larger wing primordia (García, Ramos, Sotelo, & Kondo, 2016; Hall, 2008). The nymphs are yellow-orange in color, with an outline of wings (small wings that are in formation) and a pair of compound red eyes (EPPO, 2006; García et al., 2016). The duration of the nymphal stages can be 15 days under appropriate temperature conditions of 28 °C (Martínez-Carrillo, 2016). Nymphs do not transmit HLB in the field, although they can acquire it when they feed on infected plants and then transmit it as adults (Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas [Senave], 2013).

First-instar nymphs are 0.3 mm long and 0.17 mm wide, reaching 1.6 mm long and 1.02 mm wide in the fifth instar (Tsai & Liu, 2000) (figure 2a). The color pattern of the adult is characteristic, with dark spots towards the edge of the wings and slightly transparent in the center (Hall, 2008). The adults remain perched at an inclination of 30-45° on branches and leaves (figure 2b), a feature that helps their identification in the field (Hall, 2006).

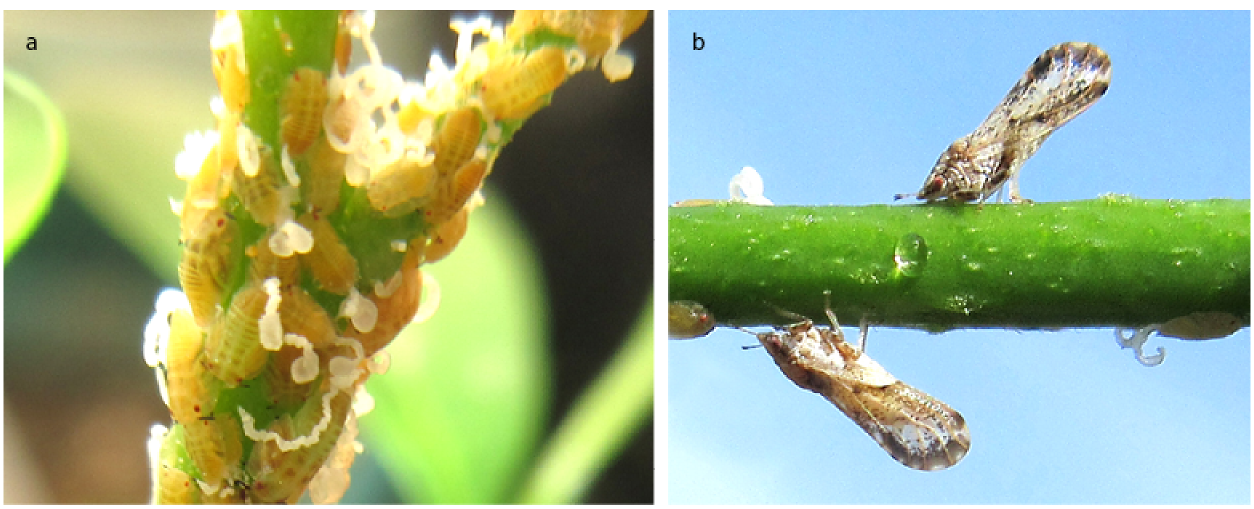

Figure 2. Asian citrus psyllid *Diaphorina citri*. a. Third to fifth-instar nymphs with their typical white and waxy excretions; b. The typical position of *D. citri* at an angle of 45°
Source: Takumasa Kondo (AGROSAVIA)

The main damage caused by *D. citri* is related to the involvement of the insect as a vector of the phloem-limited bacteria responsible for Huanglongbing disease (HLB), considered the most important and catastrophic for citrus fruits (Alemán, Baños, & Ravelo, 2007; Fonseca, Valera, & Vásquez, 2007; Halbert & Manjunath, 2004; Hall, 2008).

In the State of Sinaloa, Mexico, five species of predators associated with *D. citri* have been detected: the green lacewings *Chrysoperla comanche* (Banks) and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), the ladybug *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae) and the parasitoid *Tamarixia radiata* (Waterston) (Cortez-Mondaca, Lugo-Angulo, Pérez-Márquez, & Apodaca-Sánchez, 2009).

In the case of Colombia, a study carried out by Kondo et al. (2015) identified 16 species of natural enemies of *D. citri* distributed in six families and five orders. In Ecuador, Chavez et al. (2017) reported the presence of two natural enemies of *D. citri, Tamarixia radiata,* and *Cheilomenes sexmaculata,* which attack psyllid nymphs. For Mexico, Hernández-Fuentes et al. (2014) and Sánchez-González et al. (2015) reported close to 100 species of arthropods as natural enemies of *D. citri*, including spiders, wasps, coccinellids, chrysopids, hemipterans, and syrphids. Recently, Kondo, González and Guzmán-Sarmiento (2017), based on scientific literature, presented a list of arthropods as natural enemies of *D. citri,* comprised of 101 species distributed in nine orders and 26 families worldwide.

Causal agent: '*Candidatus* Liberibacter' spp.

In recent years, based on their 16S rDNA (ribosomal DNA) sequences, three species of *C.* Liberibacter have been isolated from trees with the disease: *C.* L. asiaticus and *C.* L. americanus (both transmitted by the Asian citrus psyllid *D. citri*), and *C.* L. africanus, transmitted by the psyllid *T. erytreae* (Bové, 2006; Gottwald, 2010). Each species of *C.* Liberibacter has evolved in the continent that gives it its name, presenting the same symptoms wherever the HLB disease develops. The authors highlight as laboratory detection methods, the dot blot hybridization with a DNA probe and various PCR formats, and using primers based on 16S rRNA or the rplKAJL-rpoBC operon sequence (Bové, 2006).

Candidatus L. asiaticus and *C.* L. americanus block vascular plant bundles (Laflèche & Bové, 1970), developing one or more yellow buds after a latent period of 6 to 18 months, while other parts of the tree remain asymptomatic (Belasque et al., 2010). Affected trees will have symptomatic as well as asymptomatic sectors, and such sectorization is prevalent in young trees (Belasque et al., 2010).

Materials and methods

The study was carried out through a technological surveillance exercise, i.e., a structured and organized process to search, capture, and analyze scientific and technological information, which allows the identification of trends and decision making. The methodology described by Orjuela-Garzón, Méndez-Arteaga and Castro (2017) was used, allowing the identification of trends and the scientific and technological development in Colombia and the rest of the world concerning HLB detection methods in the field.

The following stages were followed: initially, a key objective or question was raised to guide the entire search exercise; in this case, it was "to identify the scientific and technological trends in the early detection of Huanglongbing (HLB)." Then, the thesauri (keywords) were defined (table 1), to then proceed with the construction of two search formulas, from which the related records of scientific articles and patents in the Scopus and PatentScope databases were retrieved, respectively. These records were downloaded in flat format (structured files) to proceed with the data analysis stage (figure 3).

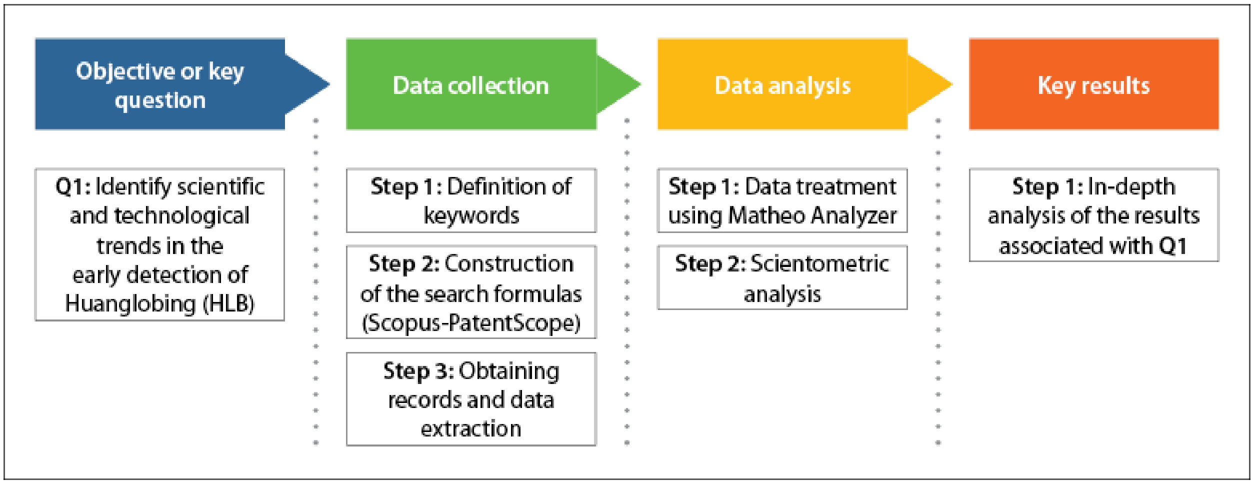

Figure 3. General methodology implemented
Source: Elaborated by the authors

For the data analysis stage, both in the case of scientific articles and patents, the information provided by the previous phase was treated through the specialized Matheo Analyzer software, version 3.2, which allows an analysis of the metrics at different levels, i.e., authors, institutions, countries, and production over time, among others, through the refined extraction of all analysis fields of the records obtained and their synthesis through graphical representations such as histograms. Matheo Analyzer allows the import of all types of structured files (bibliographic format, and format of values separated by commas CSV, among others), coming from public or private databases such as Esp@cenet, PubMed, Questel-Orbit, Dialog, STN, Scopus, and Clarivate, and easily extract particularly relevant information in fields with multicriteria or multi-information.

The structured file or flat file is imported into the Matheo Analyzer, and the extraction rules are defined; subsequently, data cleaning and grouping is carried out accordingly, to prepare the data for the scientometric analysis. In this phase, graphical representations of the metrics described above are constructed using histograms, matrices, networks or clusters; finally, the results are exported in different formats (figure 4).

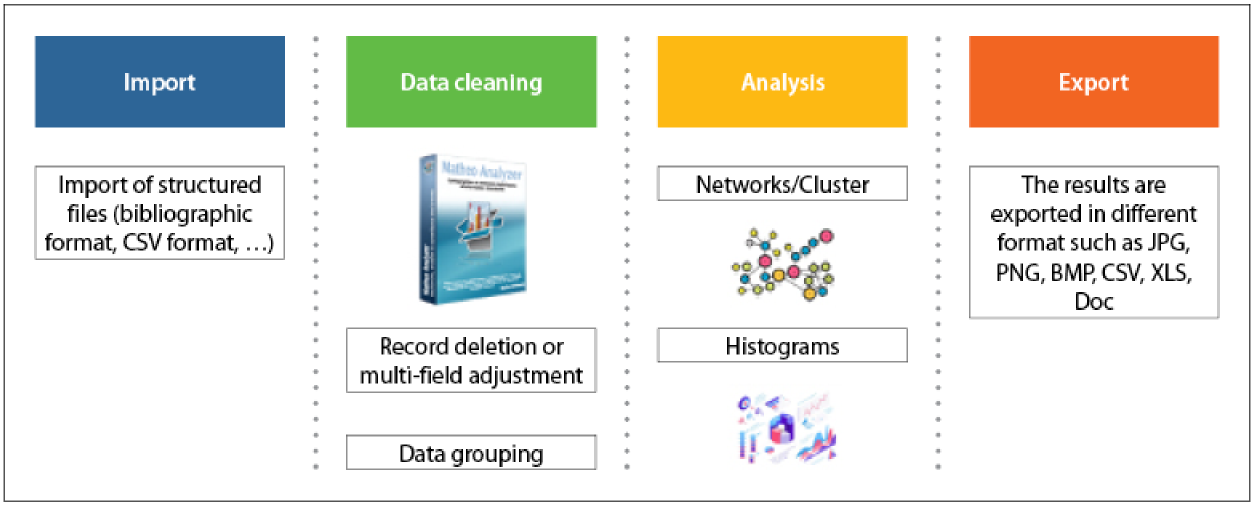

Figure 4. Data analysis process using the Matheo Analyzer software
Source: Elaborated by the authors

The search keywords or thesauri are established as a starting point (Orjuela-Garzón, Perilla-Maluche, Andrade-Navia, & Quintero-Bonilla, 2019); in this case, the thesauri were added in three groups: the first one related to the disease; the second one related to citrus fruits, and a third group aimed at the objective of the search that is its detection.

Table 1. Keywords for the search query

Group 1 Huanglongbing - "citrus greening" - "greening disease*" - "yellow shoot" - "*Diaphorina citri*" - "citrus psyllid" - "*Candidatus* Liberibacter"
Group 2 "citrus tree*" - "citrus plant*" - "Citrus fruit*"
Group 3 detection - diagnostic - identif*

Source: Elaborated by the authors

From these groups of words, a search formula for the Scopus database was designed, considering a period of 10 years (Andrade-Navia, Ramírez-Plazas, & Orjuela-Garzón, 2018). The search fields selected for the recovery of the scientific literature were Title (TITLE), Abstract (ABS), Keywords (KEY), and Publication Period (PUBYEAR). The search formula is shown in table 2.

Table 2. Search formula for scientific articles

((((((TITLE-ABS-KEY (huanglongbing)) OR (TITLE-ABS-KEY ("citrus greening")) OR (TITLE-ABS-KEY ("greening disease*")) OR (TITLE-ABS-KEY ("yellow shoot")))) OR (((TITLE-ABS-KEY ("*Diaphorina citri*")) OR (TITLE-ABS-KEY ("citrus psyllid")))) OR (TITLE-ABS-KEY ("*Candidatus Liberibacter*"))) AND ((TITLE-ABS-KEY ("citrus tree*")) OR (TITLE-ABS-KEY ("citrus plant*")) OR (TITLE-ABS-KEY ("Citrus fruit*"))))) AND (TITLE-ABS-KEY (detection) OR TITLE-ABS-KEY (diagnostic*) OR TITLE-ABS-KEY (identif*))) PUBYEAR > 2006

Source: Elaborated by the authors

In the case of patent search in the PatentScope database, a search formula was designed using the thesauri defined in table 1; besides, the search fields selected for the retrieval of records were Title (TI) and Abstract (AB) (Orjuela, Andrade, Cardona, Peralta, & Mendez, 2019). In both cases, English was used as the recovery language and was not limited by a time period. The search formula is shown in table 3.

Table 3. Patent search formula

((EN_TI:huanglongbing OR EN_AB:huanglongbing) OR (EN_TI:"citr* greening" OR EN_AB:"citr* greening") OR (EN_TI:"greening disease*" OR EN_AB:"greening disease*") OR (EN_TI:"yellow shoot" OR EN_AB:"yellow shoot") OR (EN_TI:"*Diaphorina citri*" OR EN_AB:"*Diaphorina citri*") OR (EN_TI:"*Candidatus* Liberibacter" OR EN_AB:"*Candidatus* Liberibacter"))

Source: Elaborated by the authors

Results and discussion

Scientometric analysis

The dynamics of scientific publications in a technological surveillance study plays an important role since it allows identifying trends and the degree of research progress in a given topic. In this case, 164 scientific documents were found for the 2007-2018 period, during which there is a fluctuating behavior in the number of publications, with a positive trend. This indicates that it is an area of knowledge that is still emerging. These papers were published in different journals of high impact, among which *Plos One, Phytopathology, Plant Disease, Computers and Electronics in Agriculture,* and *Journal of Agricultural and Food Chemistry* are highlighted (figure 5).

The authors that stand out belong mainly to institutions in the United States and China. Among the main ones are Won Suk Lee with eleven documents, followed by Yong Duan, with nine published documents, Reza Ehsani with eight documents, and Lukasz Stelinski with seven documents. Hong Lin, with six documents, is the only author on this list that does not belong to the United States, having as affiliation, a food safety laboratory in China (figure 5).

The most representative institutions are the University of Florida and the United States Department of Agriculture (USDA). The University of Florida has focused recently to research disease detection through precision agriculture mechanisms. On the other hand, the USDA has developed different studies for Huanglonbing detection using molecular techniques, in addition to treatment by fungicides and microbiological supplements to counteract the symptoms as well as aspects of the biology of the bacteria to understand the disease (figure 5).

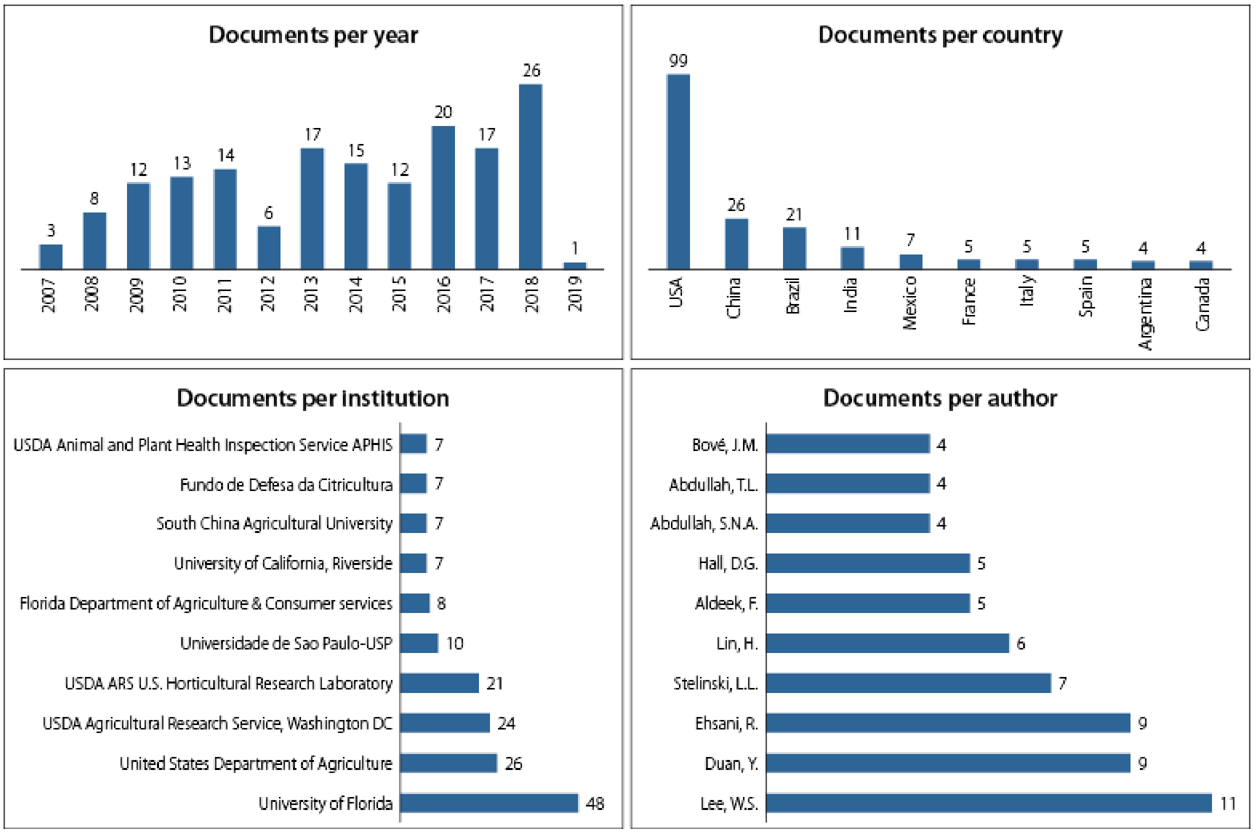

Figure 5. Scientometric analysis of HLB detection methods
Source: Elaborated by the authors

Global trends in HLB detection methods in the field

Considering that there is currently no treatment for this disease, efforts are focusing on the early detection of symptoms in trees using computerized vision systems (Cubero et al., 2016), for which the following technologies have been developed:

Laser-induced fluorescence spectroscopy (Portable LIFS-405)

This is a methodology that uses a portable laser-induced fluorescence spectroscopy system (LIFS-405) and statistical tools, capable of identifying not only HLB symptomatic leaves in the field but also asymptomatic trees (Ranulfi et al., 2016). This tool is widely used in medicine, since it can provide useful information regarding the concentration and physicochemical properties of a certain biological substrate and, eventually, it could be used as a diagnostic tool for HLB.

Laser-induced rupture spectroscopy

This is an optical method that is based on the generation of plasma through laser ablation for the spectroscopic analysis of its emission (Rao et al., 2018). The Laser-Induced Breakdown Spectroscopy (LIBS) has been initially implemented for the identification of HLB in Mexico and China, obtaining the plasma column directly from the fruit epidermis (Rao et al., 2018). The novelty of this method lies in taking fingerprints of healthy and diseased plants based on their organic and inorganic components, in addition to the use of a multi-pulse laser together with a microscope to take spectra of the plant phloem (Ponce, Etxeberria, González, Ponce, & Flores, 2018).

Fourier-transform infrared spectrometry

This technique, together with an attenuated total reflection (ATR) sampling probe, has proven effective in detecting diseases in plants (Gandolfo, Mortimer, Woodhall, and Boonham, 2016). Gandolfo et al. (2016) state that a number of factors limit the use of other regions of the electromagnetic spectrum. In the ultraviolet (UV) region (10-400 nm), solar radiation is strongly attenuated by chlorophyll and carotenoids, resulting in a strong absorption. In the near-infrared (NIR) spectral region, the high reflectance of the spongy mesophyll cells inside the leaves or the lower part of these gives rise to the appearance of strong reflection rays. In shortwave infrared (SWIR) at wavelengths between 1,200-2,500 nm, the response is strongly influenced by water content, cellulose, and lignin concentrations, as well as by other biochemical components (Curran, 1989).

When comparing healthy leaves against infected leaves in the laboratory with *C.* Liberibacter asiaticus and *C.* Liberibacter africanus and using Fourier-transform infrared spectrometry, the marked differences between healthy and diseased spectra are found in 960 cm^{-1}, 1,087 cm^{-1}, 1,109 cm^{-1}, 1,154 cm^{-1}, 1,225 cm^{-1}, 1,385 cm^{-1}, 1,462 cm^{-1}, 1,740 cm^{-1}, 2,882 cm^{-1}, 2,982 cm^{-1} and 3,650 cm^{-1}.

Raman spectroscopy

This is a non-invasive optical technique, easy to perform, and that only requires a very compact configuration, which means that it can be portable (Pérez et al., 2016). This technique measures the dispersion of inelastic light based on a monochromatic source, providing information on the chemical composition of the analyzed object (Pérez et al., 2016).

Raman spectroscopy combined with a statistical analysis of the resulting spectra, employing a principal component analysis (PCA) and a linear discriminant analysis (LDA), has shown a sensitivity of 86.9 % (percentage of positives, which were correctly identified), a specificity of 91.4 % (percentage of negatives, which were correctly identified) and an accuracy of 89.2 % (proportion of all tests that are correct) (Pérez et al., 2016). Spectral abnormalities in HLB-positive oranges and in carbohydrate-related bands, were 905 cm^{-1}, 1,043 cm^{-1}, 1,127 cm^{-1}, 1,208 cm^{-1}, 1,370 cm^{-1}, 1,272 cm^{-1}, 1,340 cm^{-1}, and 1,260-1,280 cm^{-1}.

Polarized images

Using the principle of starch accumulation in the leaves of infected citrus fruits (Pourreza & Lee, 2014), a vision sensor developed for the real-time detection of HLB in field conditions is used. The sensor detects the infection directly on the tree canopy using a highly sensitive monochrome camera (Pourreza, Lee, Ehsani, Schueller, & Raveh, 2015).

Visible and near-infrared spectroscopy (VIS-NIR) and thermal imaging

Sankaran et al. (2013) demonstrated the applicability of this technique, in addition to thermal imaging for the detection of HLB in citrus trees. Three cameras (two six-band multispectral and one thermal), mounted on the mast of a vehicle, were used to obtain the images. Multispectral cameras are configured to obtain images in 12 bands in the range of 440 nm to 990 nm. Data analysis revealed that the average reflectance values of healthy trees in the visible region were lower than those in the near-infrared region, while the opposite occurred with trees infected with HLB (wave bands such as 560 nm and 710 nm had good separability in visible near-infrared spectral regions). The average spectral reflectance in the thermal infrared region was higher in the canopies infected with HLB compared to the healthy ones (Sankaran et al., 2013).

Multiband images

García-Ruiz, Sankaran, Maja, Lee, Rasmussen and Ehsani (2013) examined aerial images of a specific area of interest, using an unmanned vehicle or drone and an aircraft equipped with a high-resolution camera, with multiband image sensors between 530 and 900 nm, adjusting the desired resolution according to the flight altitude.

The reflectance of 710 nm and the NIR index values were significantly different between healthy trees and those infected with HLB, so the authors conclude that high-resolution aerial detection has good prospects for the detection of trees infected by HLB (García-Ruiz et al., 2013).

Technological change factors in the medium and short term

Within the change factors or factors that determine the evolution of technology in the near future in terms of early detection methods for HLB, four trends of interest were identified by in-depth analysis of the 164 records obtained in the Scopus database for the 2007-2018 period: electrochemical detection, rapid molecular detection, thermal treatment and prevention using UV-blocking plastic films (table 4).

Table 4. References consulted associated with each trend

Technology category	Associated trends	References
Quick or early detection	Electrochemical detection	Volkov and Brown (2014)
	Rapid molecular detection	Rigano et al. (2014) Russell, McOwen, Bohannon, Amato, and Bohannon (2015)
Prevention	Prevention through movies	Miranda, Dos Santos, Felippe, Moreno, and Fereres (2015)
Treatment and control	Thermal treatment	Al-Jumaili and Ehsani (2015)

Source: Elaborated by the authors

Electrochemical detection

The HLB induces a very strong decrease in the amplitude of the electrical potential difference in a tree or fruit. Volkov and Brown (2014) used a non-disruptive method that consisted of a data acquisition system interconnected to a computer and identical non-polarizable reversible electrodes connected to the trees, for the measurement of electrical potential and the detection of HLB in tissue. The amplitude and dependence time of the difference in electrical potential recorded on a leaf, stem, or fruit, were sensitive to the health of the tree. Those trees that were infected have a very low direct current (DC) signal potential difference compared to the healthy ones. According to Volkov and Brown (2014), this electrophysiological method allows the rapid detection of HLB and could be used for the detection of other diseases in plants.

Rapid molecular detection

Rigano et al. (2014) developed a study in which a DNA amplification technique known as Loop-mediated Isothermal Amplification (LAMP) was adapted for the detection of *C.* L. asiaticus. This methodology was combined with a lateral flow rod device for the visual detection of the resulting enlargements, eliminating the need to perform agarose gel electrophoresis to visualize the result. The assay was highly specific for the bacteria of interest. By serial dilution of purified DNA from an infected plant, the sensitivity of the assay was found to be 10 picograms. Additionally, this level of sensitivity was similar to the values obtained by running a real-time PCR in parallel (Rigano et al., 2014). This methodology was able to detect *C.* L. asiaticus from different types of samples, including infected citrus plants and psyllids (Rigano et al., 2014).

HLB is a disease that has a long incubation period, during which the pathogen is in low concentration and is not systematically distributed (Gottwald, 2010), making diagnosis and eradication difficult (Bayer, 2015).

Russell et al. (2015), as researchers of the Agdia company, have developed a new and fast molecular technique, AmplifyRP®, which allows detection at the PCR level in minutes and also in the field. AmplifyRP® uses a methodology based on polymerase recombinase for the amplification of DNA at a single temperature. In contrast to conventional or real-time PCR, AmplifyRP® has no requirements for DNA purification, nor thermocycling. The results can be read using small and easy-to-use devices, in addition to eliminating the need for expensive PCR equipment and technically trained personnel, and reducing the number of chemical reagents (Russell et al., 2015).

Given its characteristics, AmplifyRP® is reinforced as an ideal tool to monitor the progression of HLB by early detection of *C.* Liberibacter spp. in citrus trees or insect vectors (Russell et al., 2015).

Thermal treatment

Al-Jumaili and Ehsani (2015) developed a mobile thermal treatment mechanism that consisted of a tree coating system attached to a citrus transport truck, a portable steam generator, a water tank, a water supply pump, and an electric generator. The plot is covered with a relatively heat-resistant opaque plastic tarpaulin to completely cover an area of approximately 14 m^3 in volume (Al-Jumaili & Ehsani, 2015). The steam is supplied from a portable generator, which is loaded in the back of the transport truck, and the trees are vaporized at different temperatures and in different durations, from 0-6 min (Al-Jumaili & Ehsani, 2015). The authors indicate that the device is simple in design and efficient for use in the field, although they warn that the long-term effect of heat treatments is still under investigation.

UV blocking

The vision, behavior, and performance of insect pests can be manipulated using blocking materials (UV). Therefore, Miranda et al. (2015) developed a study to evaluate how plastic films that block UV rays could affect the takeoff and the localization capacity of *D. citri* of the host plant. Adult psyllids were released from a vial into a seat covered by a UV or standard (control) blocking film to assess this effect, and the number of insects remaining in each vial under each treatment was counted at different time intervals (Miranda et al., 2015). The authors observed that the localization capacity of *D. citri* of the host plant is interrupted in a UV-deficient environment (Miranda et al., 2015). In this way, the results show that UV blocking materials could become a valuable strategy for the integrated management of *D. citri* and HLB in citrus plants cultivated in closed environments (Miranda et al., 2015).

Technological development progress in HBL

The PatentScope database was explored using a search formula shown in table 3, and finding that 234 patents have been registered for all available years. The registration dynamics over the years showed a constant growth, being 2017 the year with the highest number of patents (58). These were registered mainly in China and the United States, and via the Patent Cooperation Treaty (PCT), with Huaichun Li being the main inventor with patents.

The review showed that the three main institutions that hold patent rights are Bayer Crop Science, the Research Foundation of the University of Florida, the Valencian Institute for Agricultural Research, and the Agricultural University of southern China (figure 6).

Finally, when reviewing the different patent families (subclasses) according to the International Patent Classification (IPC), the main areas under which inventions have been patented concerning HLB, are pesticides, biocides, antimicrobial compounds and the use of enzymes and microorganisms (genetic engineering) to prevent the proliferation of unwanted organisms; the areas with the highest number of records are A01N 57/12, A01P 1/00, and C12N 15/82.

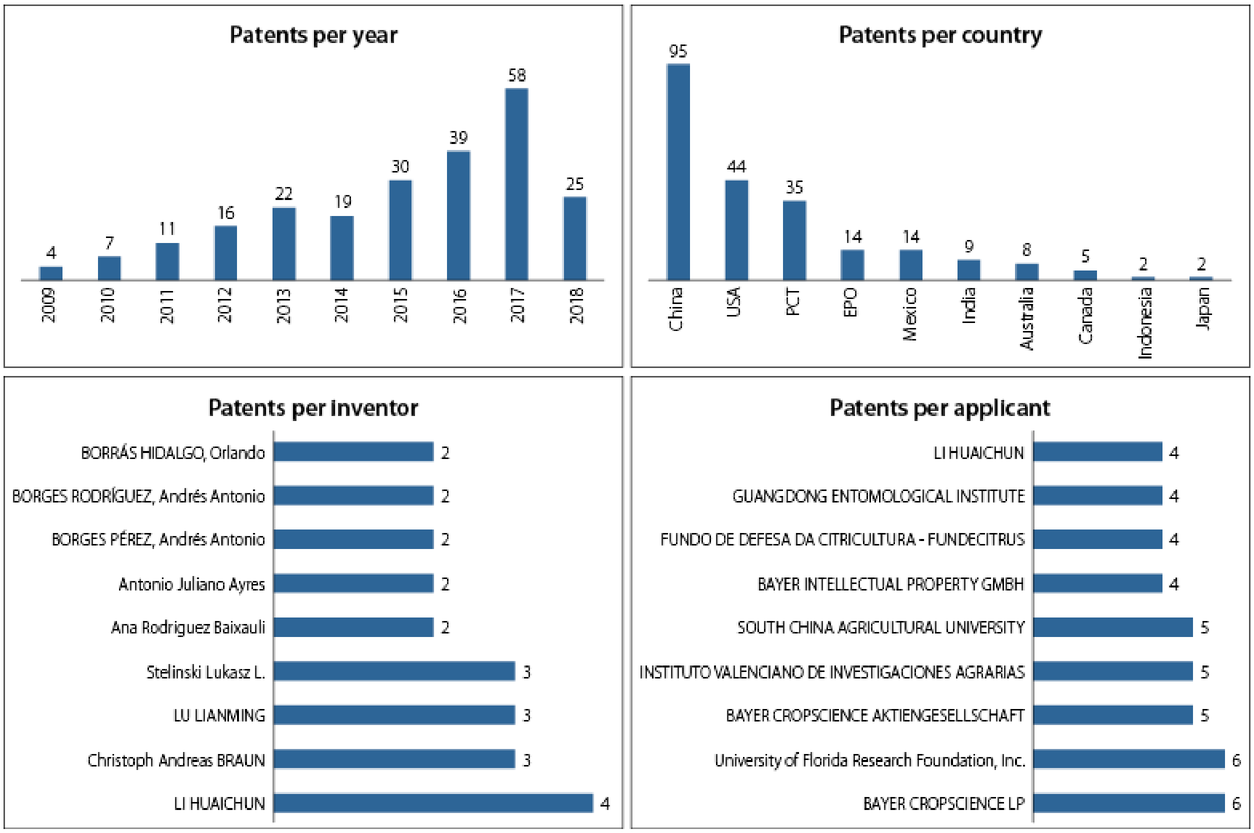

Figure 6. Analysis of the technological development of HLB
Source: Elaborated by the authors

Outstanding patents

LAMP detection method for the rapid detection of Candidatus Liberibacter asiaticus

Applicant: Inspection and Quarantine Comprehensive Technology Center, Jiangxi Entry-Exit Inspection and Quarantine Bureau.

Inventors: Huang Lili, Li Yin, Zhu Jianxin, Luo Taopeng, and Liu Ying.

The invention CN105524986 (2015) describes the LAMP method for the rapid detection of *C*. L. asiaticus. This method comprises the following steps: (1) design of four specific LAMP primers by adopting 16S rDNA from *C*. L. asiaticus as the target gene; (2) extraction of total nucleic acid from the citrus leaf by adopting the CTAB technology; and (3) detection of the specificity of the LAMP method.

The method has the advantages of being simple, easy, and economical, since it requires few devices, in addition to being fast and accurate. It is especially suitable for large-scale primary screening of *C.* L. asiaticus, providing a new form of detection and control.

Field detection method for the citrus greening disease in citrus in the near-infrared spectrum

Applicants: South China Agricultural University

Inventors: Zeng Xinnian, Chen Dongmei, and Wang Huatang

The invention CN106018332 (2016) describes a field detection method for HLB in the near-infrared spectrum. The method includes the following steps: (1) a near-infrared portable spectrometer is adapted to detect and read directly near-infrared spectroscopy data from citrus leaves infected with bacteria and plants without the disease in the field; (2) establishment of a qualitative classification judgment model: the near-infrared spectroscopy data in stage 1 is subjected to correction and preprocessing, and then the characteristic values of its near-infrared spectroscopy are extracted, and a qualitative evaluation model for the classification of partial minimum squares (discriminant analysis) (PLS-DA) is established; (3) application of the qualitative classification method to unknown samples: the A model established in step 2 is used to distinguish whether citrus leaf samples were infected or not with the bacteria in the field. Only 3 to 5 leaves are needed for scanning, and rapid diagnosis can be achieved in just 1 or 2 minutes. In addition to being simple, fast, and accurate, this method does not cause any damage to the samples and is environmentally friendly.

Conclusions

The first step for the effective control of the HLB spread is the early and accurate detection of the disease. Precision agriculture tools such as automated vision systems are promulgated as alternatives of great potential for the permanent monitoring of crops from planting to harvest. Such systems can be used to detect citrus trees infected with HLB, as long as some parameters such as multiple measurements per tree and the selection of the appropriate spectral band are considered. However, the evaluation of symptoms presents an enormous difficulty in their early detection, since a plant can take up to six months or more to show HLB symptoms (Belasque et al., 2010).

The United States and China are the countries that have the highest interest in the subject, the first of which has contributed to the largest number of scientific documents (99 scientific articles). Furthermore, China also owns the largest number of patents (95 patents). Within this review, the University of Florida and the USDA stand out as the leading research institutions in this topic.

The University of Florida has focused on early detection issues of the disease, through precision agriculture mechanisms in recent years, which has allowed HLB to be detected non-invasively in the field. This reflects the special interest of this institution in developing this research area.

The technological production of Bayer Crop Science as a leader in patent development for commercial purposes consists of products to reduce bacterial infections in citrus fruits. In the scientific production of

Latin America, Brazil, Mexico and Argentina stand out with 21, 7, and 4 scientific articles, respectively. For Colombia, two scientific articles related to DNA extraction methods and real-time PCR validation for the detection of HLB are reported.

In the molecular area, research topics are developed in detection through DNA amplification by PCR, which is the most used technique in the diagnosis of HLB. It is from this technique that other investigations have been developed, which allow obtaining a reliable and faster result that requires highly trained personnel. Some of the proposals are the use of molecular probes and isothermal amplification using AmplifyRP®.

The application of polarized light methods can offer an economic detection tool. However, its application in Colombia has to be considered due to the presence of physiopathies (Ríos-Rojas, Correa, Rojas-Marín, & Dorado-Guerra, 2018) that show a spotting on the leaves similar to the symptom produced by HLB, making it difficult to use these remote sensing methodologies. Therefore, the biochemical changes that influence the absorbance, transmittance, and reflectance of light should be clearly defined for plants with severe water stress and those with the physiopathy in question, and in contrast, what is reported in the literature related to HLB. This may be possible as long as the type of sensor and the spectral responses that can differentiate between these diseases are defined.

On the other hand, in addition to early diagnostic techniques, the development of strategies related to vector monitoring, as well as the distribution of hosts, is recommended. This task can be supported by Geographic Information Systems that offer, in addition to geolocation instruments, spatial tools that correlate variables and allow decisions to be made during monitoring and sampling.

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Disclaimers

All authors made significant contributions to the document and agree with its publication; further, they state that there are no conflicts of interest in this study.

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