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Agroclimatic risk of development of *Diaphorina citri* in the citrus region of Nuevo Leon, Mexico

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Diaphorina citri **is the insect vector of the disease, Huanglongbing (HLB) which is the most devastating citrus disease worldwide. It is necessary to identify potential risks; this can be done based on favorable thermo-pluviometrical conditions for the vector. The present study aims to identify potential risks in the Nuevo Leon citrus region in areas, which have not yet been devastated by the citrus disease. The presence of HLB and the population growth of the *D. citri* will be influenced by four factors involved in the epidemiological system: the disease-prone host - citrus fruits; the pathogen agent; the biology of the vector; and the climate conditions that foster the vector establishment and reproduction. With information from 65 weather stations, some indices were estimated: potential generations of the vector per year, days with favorable temperature and suitable rainy days for the vector, area planted with citrus fruits, and finally, the potential risk index. This shows that the Nuevo León citrus region is a HLB free region. The method can be applied to citrus producing areas which have not been devastated by the citrus disease worldwide.**

Key words: Citrus greening disease, climate conditions, Huanglongbing, potential risks.

INTRODUCTION

Of the worldwide production of citrus fruits, the bulk is achieved in eighteen countries that together produce 115.5 million tons (FAOSTAT, 2014). Production has been affected by pests and diseases, including the deadly bacterial disease to the vector Huanglongbing (HLB), also known as citrus greening disease, which is considered to be the greatest threat to citrus cultivation worldwide. The agents are Gram-negative bacteria of the genus *Candidatus* Liberibacter, which includes three species: *C.* Liberibacter africanus, *C.* Liberibacter americanus and *C*. Liberibacter asiaticus; the last named is the most widely distributed species in Asia and America because of its tolerance of high temperatures (Bové, 2006; Narouei et al., 2016). HLB is restricted to the phloem of the host citrus plant, and in citrus crop, it is transmitted by vegetative means such as grafts. In nature, it is transmitted by the psyllid insects *Triozaerytreae* and *Diaphorina citri*, the latter having been first detected in China more than 100 years ago (da Graça et al., 2008).

Until 2003, HLB was restricted to two continents, Africa and Asia. It was reported on the American continent for

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the first time in 2004, in Araracuara, São Paulo, Brazil, where the presence of *C.* Liberibacter asiaticus was detected (Colleta-Filho et al., 2004); in 2005 its presence was confirmed in Florida, USA, followed by Cuba in 2007 and Louisiana, USA in 2008.

The disease affects various links in the chain of citrus production, thereby reducing employment opportunities in harvesting, manufacture of machinery and boxes, and transport and final consumer price of the fresh fruit. In addition, it destabilizes industries engaged in processing the product: producers of juices, concentrates, oil extracts and dried peel, and the transport and final consumer price of these.

The ability of the Asian citrus psyllid (ACP), *Diaphorina citri*, to transmit HLB constitutes a serious threat. This small insect feeds on the leaves and stems of citrus trees and related species such as jasmine and curry leaves. It can be spread by anthropogenic factors such as the commercial and tourist activities using aircraft, ships, trucks and cars, and also by anemochorous dispersion, like the wind.

Citrus production in Mexico ranks fourth worldwide, after Brazil, China and the USA. There are over 334,658.68 ha across twenty-three States in Mexico, which together account for an annual production in excess of 4 million tons of fruit, with an approximate value of US 461 million dollars in 2012.

HLB was first recorded in 2009 in the states of Yucatan and Quintana Roo, owing to the proximity to, and trade with, Cuba, the USA (Florida and Louisiana) and the Dominican Republic. Investigation has been scarce and isolated: Lopez et al. (2009) noted the lack of a master research plan and recommended the activation of an emergency plan. The memorandum NOM-EM-047-FITO-2009 from the national body for health and food safety in agriculture established phytosanitary actions leading to the implementation of a monitoring program that includes sampling, diagnosis, inspection and monitoring for the timely detection of the introduction and spread of HLB in Mexico, as well as the application of phytosanitary measures required for controlling it, including the delimitation of areas under phytosanitary control, disposal of infected materials, vector control, sampling inspection and restriction and/or control of the movement of plant material from the host species (Diario Oficial de la Federación, 2009).

The disease was expected to spread gradually towards citrus-producing areas on the slopes bordering the Gulf of Mexico; however, in April 2010, the bacterium made a turn; it was detected in Mexican lemon trees in the municipality of Tecoman, Colima, a state bordering the Pacific. To date, the disease has been detected in 13 of Mexico's 23 citrus production states (SENASICA-DGSV, 2014).

There is yet no cure for the affected trees. Based on current regulations and experiences in other citrus regions of the world, the strategy for managing HLB includes disposal of infected material and, keeping orchards productive as long as possible, learning to live with the disease, and applying strict vector-control methods.

The presence of HLB in Africa, Asia and America led to studies on its economic and social impact in affected countries; in all cases, the results point to huge losses associated with the disposal of infected trees and the rise in production costs due to management practices to prevent the disease (Taylor et al., 2016).

Diaz et al. (2014) focused on the environmental conditions that foster the presence, abundance and population growth rate of *D. citri*. Other lines of research have developed indices to delimit agroclimatic risk areas (Moschini et al., 2010).

Areas not affected by the disease include the Nuevo Leon citrus region, an area recognized as the birthplace in Mexico for the cultivation of the orange (*Citricus sinensis*): the first juice-processing plants in Latin America were established there. Orange production in 2014 was 204,750 tons; Nuevo León ranked fifth at national level.

However, some areas have not yet been affected by HLB, even some that are contiguous with affected areas. It is necessary to identify potential risk, and this can be based on thermo-pluviometrical conditions favorable to the vector. The present study aims to identify potential risk for areas in the Nuevo Leon citrus region, a region that is not yet affected by the disease.

MATERIALS AND METHODS

HLB is fostered when temperature, vector insects and the developmental stage of citrus trees combine and interact. This agrosystem, generically known as an epidemiologic triangle (Bové, 2006; Batool et al., 2007; da Graça et al., 2008) has served in the present study as a basis for considering additional environmental conditions: rainfall, relative humidity, photoperiod and insect dispersion by large air masses. Consideration of all these parameters together has allowed the configuration of the Pathosystem Tetrahedron (Figure 1).

In this case, the disease-prone host is the orange tree, the pathogen agent is the *Candidatus* Liberibacter asiaticus (CLas), the vector is *D. citri*, and the environmental conditions are primarily climatic ones (temperature, precipitation, relative humidity, photoperiod and the dynamics of large air masses).

In order to determine each of the factors involved in this tetrahedron, the literature on the environmental conditions (climate) that favor each life stage of *D. citri* was reviewed (Yang et al., 2006; Bové, 2006; Batool et al., 2007; Diaz et al., 2014; Moschini et al., 2010). The environmental conditions in the study region were ascertained from analysis of the climate records of 65 stations included in the Computerized Climate System database (CLICOM) of the National Meteorological Service (SMN, 2014). The data used included daily maximum and minimum temperatures and daily precipitation. The reliability of these data was tested by path and homogeneity tests. The average climatic values were also obtained from SMN. Statistics of the planted and harvested areas of the various citrus fruits were obtained from the national agricultural information system.

The concept of potential generation of the vector, refers to the number of generations that can be produced, according to the

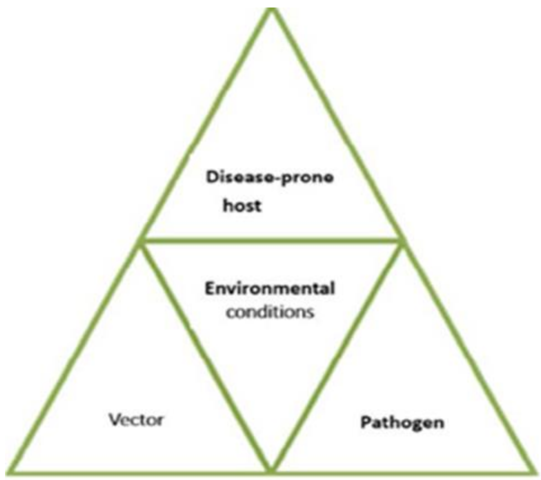

Figure 1. Pathosystem tetrahedron.

biology of the vector and the environmental conditions. One generation of *D. citri* requires 211 degree-days to complete its development, with a threshold temperature of 13.5°C (Diaz et al., 2014; Moschini et al., 2010). The mean daily temperature of each station was used here to calculate the degree-days (GD), at each station.

$$GD_i = GD_{i-1} + (Tmed_i - 13.5)$$

Where, $Tmed_i$ is the average of the maximum and minimum temperature for each day at that station, and 13.5°C is the baseline.

$$Tmed_i = (T\max_i + T\min_i)/2$$

When the cumulative value is 211°-days, one generation of the psyllid would have been completed. Once the potential number of generations has been calculated, the Potential Generations Index (PGI) is calculated for that station:

$$PGI_i = PG_i / Max(PG)$$

where: PGI_i : potential generations index for the *i*-th station; *PGi*: is the number of potential generations calculated for that station; *Max (PG)* is the maximum number of potential generations observed for all stations.

The number of days with favorable temperature conditions refers to the number of days with temperatures favoring a more rapid development of *D. citri* eggs, nymphs and adults. The number of days that meet the condition of minimum temperature, 15°C and maximum temperature, 32°C were identified, this being the temperature range reported to foster the development of the vector (Moschini et al., 2010). Once the number of days for each station has been determined, the index of days with favorable temperature conditions is calculated as:

$$IDFC_i = DFC_i / Max(DFC)$$

Where, $IDFC_i$ is the index of days with favorable temperatures for the *i*-th station, DFC_i is the number of days with favorable temperatures for the *i*-th station, *Max (DFC)* is the maximum number of favorable days observed for all the stations.

With the precipitation data obtained for each station and considering the rainfall requirement of more than 50 mm for the vector insect according to Moschini et al. (2010), the number of days that meet this requirement, that is, the number of days with favorable precipitation is calculated as follows:

$$IDFP_i = DFP_i / Max(DFP)$$

Where, $IDFP_i$ is the index of number of days with favorable rainfall for the *i*-th station, DFP_i is the number of days with favorable conditions for the *i*-th station, *Max (DFP)* is the maximum value of favorable days observed for all the stations.

The maximum orange-planted area known at the municipal level defined as Index of Planted Area, was calculated according to the following formula:

$$IPA_i = PA / Max(PA)$$

Where, IPA_i is the index of the area planted with citrus recorded for the *i*-th station, PA_i is the area planted with citrus recorded for the *i*-th station, *Max (PA)* is the maximum area planted with citrus observed for all stations.

Based on the average indices previously calculated, the potential risk index to identify areas at risk of a potential impact of *D. citri*, the HLB vector, was estimated as:

$$RI_i = PGI_i + IDFC_i + IDFP_i + IPA_i / 4$$

where: PGI_i Potential generations index for the *i*-th station, $IDFC_i$: index of days with favorable conditions for the *i*-th station, $IDFP_i$: index of days with favorable precipitation for the *i*-th station, IPA_i: index of area planted with citrus recorded for the *i*-th station.

The calculated indices were grouped in a database associated with weather stations, which are georeferenced. These data were exported to a Geographic Information System (GIS). ArcGis software 10 allowed the database to be linked to a digital map; this software contains the Geostatistical Analyst Tool, which performs spatial interpolation processes. The study area lies in the central west portion of the State of Nuevo Leon (24° 30' − 25° 30' N; 99° − 100° 20' W). It includes the municipalities of Allende, Cadereyta Jimenez, General Teran, Hualahuises, Montemorelos, Linares and Rayones. The total citrus area amounts to 25 589 ha (Figure 2). Because of the geographical location, altitude above sea level (between 300 and 1,300 m) and dominant meteorological phenomena across the citrus-growing region, the prevailing climate is semi-warm subhumid, (A)Cw, with temperatures between 18 and 22°C and summer rainfall, and small areas with a regime intermediate between summer and winter, (A)Cx'w.

In general, rainfall was not abundant. In low-altitude land, annual precipitation ranges from 400 - 600 mm. Most of the citrus region receives 600 - 800 mm, and only in the small areas does annual rainfall fluctuate between 800 and 1 200 mm.

RESULTS

In the study area, temperatures ranged from -2 to 40°C. Minimum temperatures varied between -2 and -8°C from October to February but rose thereafter, exceeding 10°C from May to August. Maximum temperatures exceeded 35°C and rose towards midsummer, reaching a peak of 40°C in May. Both minimum and maximum temperatures are limiting factors for the vector, since it is intolerant to

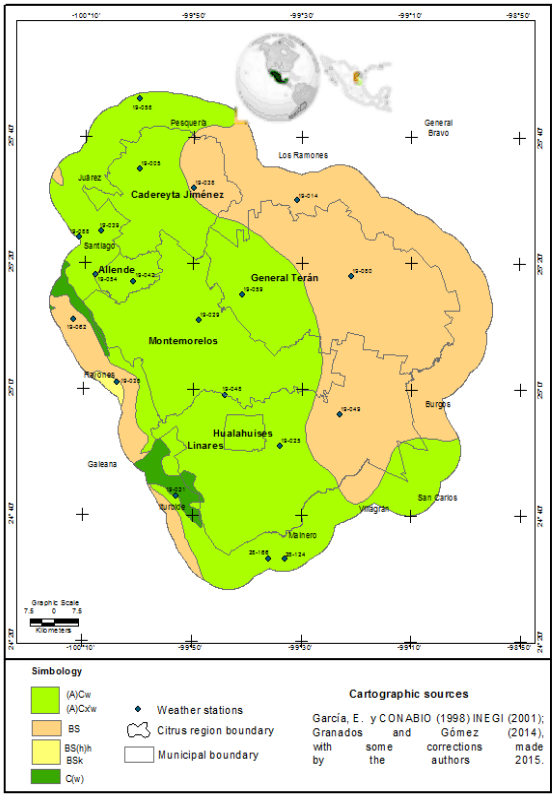

Figure 2. Climate across the Nuevo Leon citrus region. Inset: Location in Nuevo Leon State.

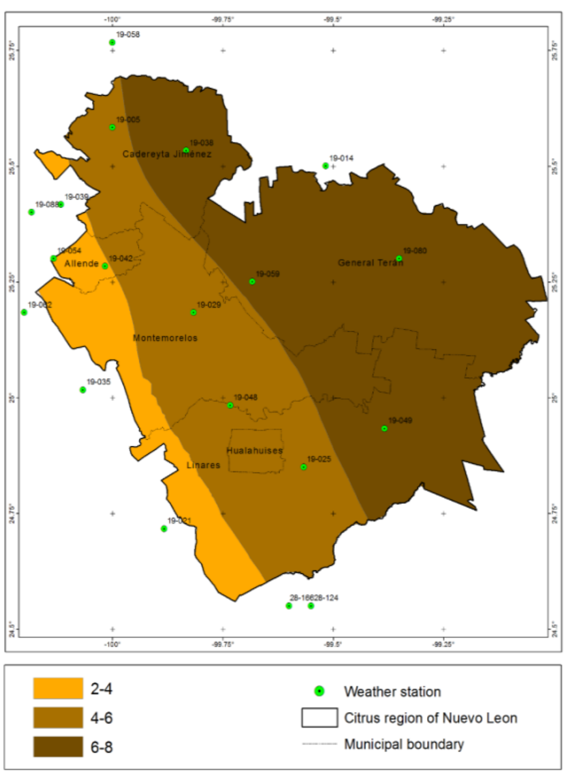

Figure 3. Potential generations of *D. citri* per season.

extreme conditions. The relative humidity is variable, being above 60% from May to September when monthly precipitation exceeds 50 mm. September is usually the month with the highest precipitation; the average value recorded for that month was 105 mm.

The derived number of potential generations indicated that between 2 and 8 generations of *D. citri* may be developed per year in the citrus region (Figure 3); 2−4 on the outskirts of the Sierra Madre Oriental, a temperate area with temperatures between 12 and 18°C (western portion of the Allende, Montemorelos and Linares municipalities); 4−6 in important areas of Cadereyta Jimenez, Montemorelos and Linares; and 6−8 towards the Gulf Coastal Plain, where warm conditions prevail (22−24°C), corresponding to the eastern portion of Cadereyta Jimenez and Linares, and the entire General Teran.

The mean number of favorable days for the development of the vector in the citrus region ranges from 21 to 90 days per year: 61−90 days in General Teran and the eastern portion of Cadereyta Jimenez and Linares municipalities; 41−60 days in Hualahises and the central portions of Cadereyta Jimenez, Linares and Montemorelos; and 21−40 in Allende and the western portions of Montemorelos and Linares, areas in the vicinity of the Sierra Madre Oriental where the altitudinal gradient leads to a drop in temperature (Figure 4).

The highest number of days with favorable precipitation for the development of the vector occurs in the central portion of the citrus region. This central area receives 400 to 600 mm of rainfall per year, exceeding the 50 mm on only 117 days per year. The region's western portion received more than 50 mm of rain during up to 78 days per year and the respective index ranged between 0.1 and 0.4. In General, Tera, and northeastern Cadereyta Jimenez, Montemorelos and Linares, the rainfall exceeded 50 mm on 17 - 39 days per year, and here the index is also 0.1 – 0.4 (Figure 5).

Orange plantations comprised between 1,000 and 40,000 ha in the citrus municipalities (Figure 6). The municipality with the largest area with oranges was General Teran, with 39 231 ha, followed by Linares, Cadereyta Jimenez, Montemorelos, Allende and Hualahuises, respectively. Then, some municipalities may experience more economic consequences than others.

The risk index (Figure 7) indicated the risk level associated with the development of *D. citri* and the potential incidence of HLB in the citrus region. The municipalities of General Teran, Cadereyta, Montemorelos and Linares have a high potential risk, with an index that ranges between 0.61 and 0.90. There is a lower risk to the west region, with an index of 0.21- 0.60.

DISCUSSION

From the detection of the disease in citrus plants in

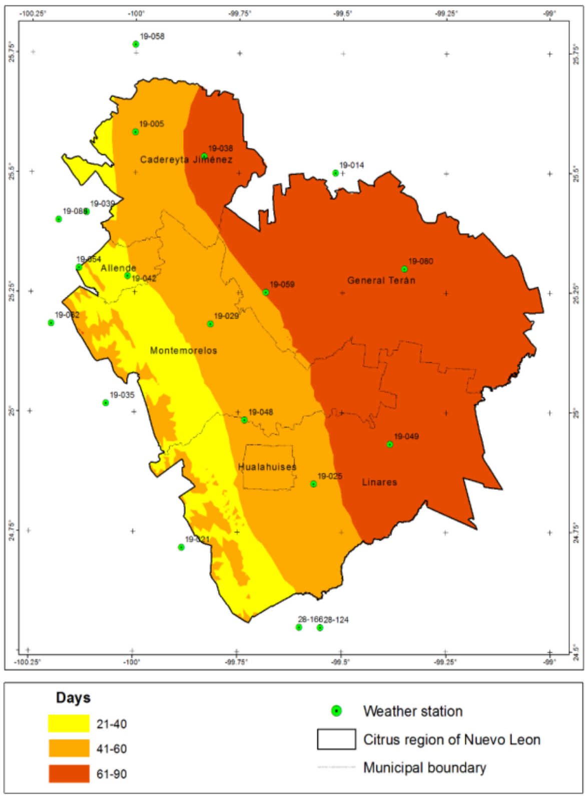

Figure 4. Number of days with temperatures favorable to *D. citri.*

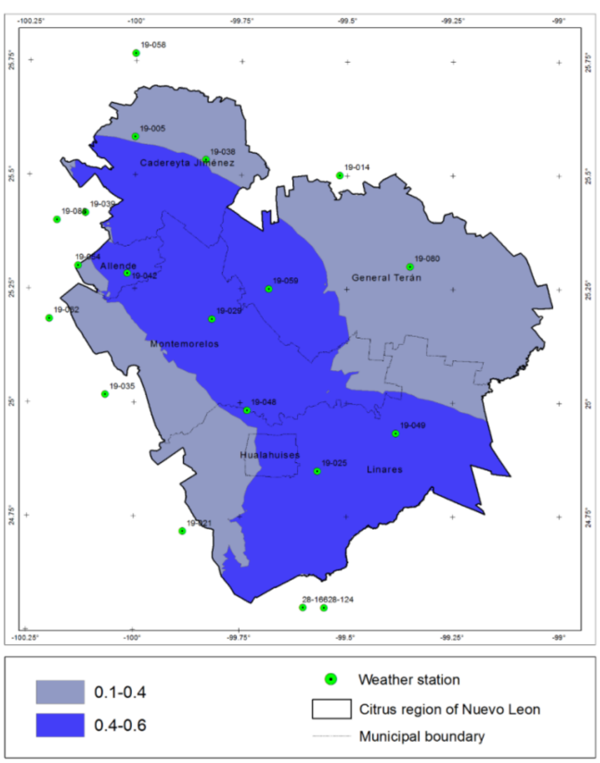

Figure 5. Index of days with rainfall favorable to *D. citri.*

America in 2004, research works on the biology of *Diaphorina citri,* the disease vector, have increased (Halbert and Manjunath, 2004; Narouei et al., 2016). Grafton-Cardwell et al. (2013) stated that climate exerts a number of direct effects on the life cycle and reproduction of *D. citri*.

Although, in Mexico, the presence of HLB was detected a little later, research on this topic was also conducted (Lopez et al., 2009), but no cure was found for HLB to date. It has been suggested that the affected areas should adopt an integrated and appropriate strategy, including the elimination of diseased trees, use of non-contaminated plants, pesticide application and biological control of the psyllid vector. Investigations are currently ongoing, and Tabachnick (2015) has stressed that the search for further solutions to reduce the devastating impact on citrus fruit production is urgent.

This search should also include research focused on disease-free citrus production areas, to diagnose the potential risk, such as studies that include the geo-technologies: spatial modelling, simulation of risk areas and map algebra (Magarey et al., 2007; Ladányi, 2010; Gutierrez et al., 2011; Rosa et al., 2011; LANGIF, 2015; NAPPFAST, 2015; Narouei et al., 2016; Taylor et al., 2016).

Investigations at a country scale were carried out by Torres-Pacheco et al. (2013), and delimited potential regions and degrees of risk based on the environmental affinities of *Diaphorina citri*, as well as the range and phenology of host plants. In southeastern Mexico, a region characterized by high temperatures (> 25°C) and precipitation (>1100 mm), the potential existence of up to 27 generations were quantified.

On a regional basis, in northern Mexico, the potential distribution of *D. citri* in Nuevo Leon was analyzed, based on the synchrony between phytophage and host, by calculating and determining the spatial distribution of specific temperature and precipitation indicators (days degree and days with high probability of rainfall, respectively) throughout the year. The most vulnerable areas were located in the warm (22 - 24°C air temperature) and semi-dry (400-600 mm precipitation) Gulf of Mexico highland, which includes the eastern area of Cadereyta Jimenez and Linares and the entire General Teran municipalities; in this area, the phytophage may put the Nuevo Leon citrus plantations and the industry at high risk within just 6-8 generations.

Prevention-oriented research contributes to scientific information that will allow the development of actions to reduce vulnerability to this citrus disease (LANGIF). The information derived from this work may be regarded as preventive and represents an instrument that will support the planning of work schemes and the proposal of new courses of action aimed at reducing the effect of this disease. Besides, key stakeholders should be involved, including specialists in natural areas, socio-economic disciplines and leading producers who promote new

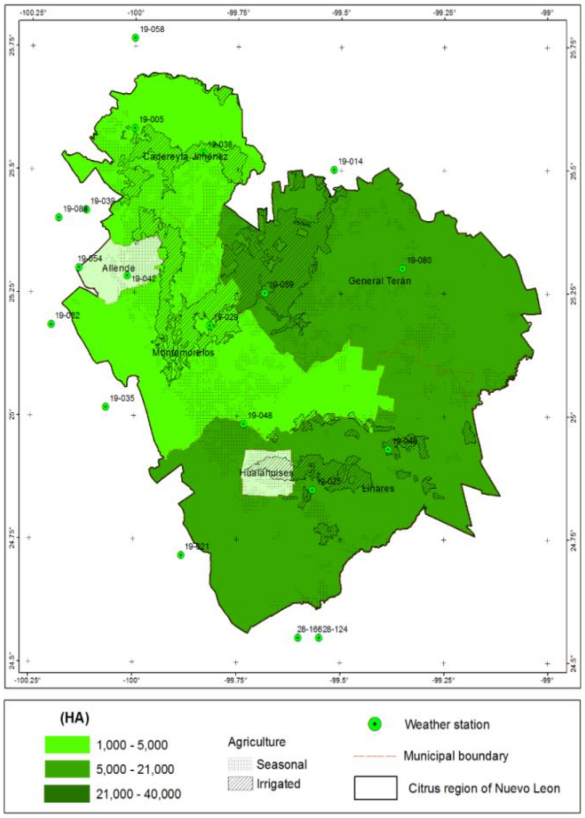

Figure 6. Index of the agricultural area.

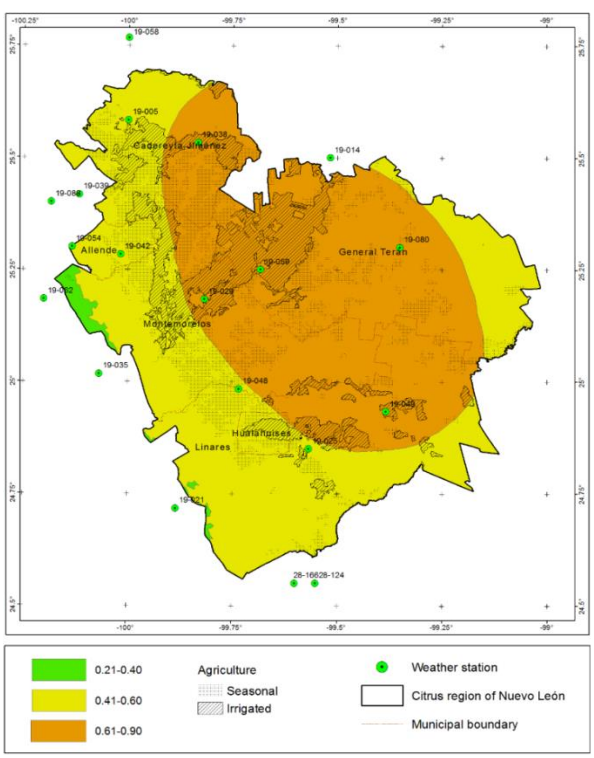

Figure 7. Risk index for the development of *D. citri* in the Nuevo Leon citrus region.

agricultural approaches for these plantations.

Conclusions

The spatial representation of phytosanitary risk, integrated from the analysis of the main thermo-pluviometric variables quantified here, allowed the evaluation of potential danger of vector, *D. citri* establishment in this region. Although, non-vector psyllids were present, conditions in the study area were seemingly suboptimal for the development of the vector; accordingly, the Nuevo Leon citrus region is free of the danger of HLB. The most important climatic element was the distribution and variation of temperature, which sometimes exceeded 40 or fell below 3°C. Lower temperatures would hinder the establishment and development of the psyllid population by slowing down its metabolism and, hence, disrupting embryonic development. The maps show this to be particularly applicable in the western part of the Nuevo León citrus region, where the development of the vector is limited by temperature extremes associated with altitude and by the smaller areas planted with the host trees.

The infected production areas are remote from this region, but the dispersal of psyllids carrying HLB cannot be ruled out, since this may be encouraged by atypical atmospheric phenomena such as variations in wind speed and direction, and incident light. Further analysis of these phenomena should produce more comprehensive indices and thereby improve the spatial representation of risk. The anthropogenic components must be strictly controlled, as established by the phytosanitary standard, NOM-EM-047-2009, in order to prevent the transport of infected specimens into the study region.

Conflict of interests

The authors have not declared any conflict of interest.

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