INCIDENCE OF GREENING IN THE REGION OF ARARAQUARA – SP

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SUMMARY

Huanglongbing (HLB) or greening is currently considered the disease with the greatest destructive potential in the world's citrus industry. Brazil is a large citrus producer, and HLB harms and puts at risk the entire citrus production chain in the country, causing serious losses. Characteristics of this disease include the loss of symptomatic fruits and reduced quality and productivity. The dispersal capacity of the insect vector, the psyllid *Diaphorina citri*, between peripheral areas (borders), in addition to the ability to spread this pathogen by insects developed on contaminated plants, it requires organization to manage the disease control, making such an act very difficult. In this sense, the present work aimed to evaluate HLB indices in the total area of properties in the region of Araraquara, SP. Inspections were carried out on the reports provided by Fundecitrus and the data obtained was subjected to descriptive analysis to assess the frequency of occurrence of the disease. As a result, it was observed that sampling research is extremely important and efficient in combating the disease, especially at the beginning of its appearance, allowing us to conclude that it is control of the disease is possible when management is adequate.

Key words: Citrus;*Diaphorina citri*; Illness; Psyllid.

ABSTRACT

Huanglongbing (HLB) or greening is currently considered the disease with the greatest destructive potential in citriculture worldwide. Brazil is a large citrus producer, and HLB harms and puts the entire production chain of the crop in the country at risk, causing serious losses. The fall of symptomatic fruits and reduced quality and productivity can be highlighted as characteristics of this disease. The ability of the insect vector, the psyllid*Diaphorina citri*, to disperse between peripheral areas (borders), in addition to the ability of this pathogen to spread by insects developed on contaminated plants, demands organization for the management of disease control, making such an act very difficult. In this sense, the present work had as objective to evaluate the HLB indexes, in the total area of properties in the region of Araraquara, SP. Inspections were carried out on the reports provided by Fundecitrus and the data obtained were submitted to descriptive analysis to assess the frequency of occurrence of the disease.As a result, it was observed that sampling research is extremely important and efficient in combating the disease, especially at the beginning of its onset, allowing to conclude

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that is possible to control this disease when the management is adequate.
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1. INTRODUCTION

Brazil is one of the largest citrus producers in the world, where estimated production per harvest averages 364.4 million boxes of 40.8 kg. The state of São Paulo, from this perspective, is mainly responsible for this estimate of national production (FUNDECITRUS, 2017). However, in contrast to the speed at which the national citrus industry develops and grows, numerous pests and diseases appear that cause direct and indirect damage, as they are insect vectors of phytopathogens (PARRA et al., 2003; GRAVENA, 2005). Regarding diseases, the one that has the greatest economic impact is huanglongbing (HLB) or greening, caused by the bacteria *Candidatus liberibacter* spp. are being considered the most important for the global citrus industry (BOVÉ, 2006).

The first observed cases of HLB in Brazil were observed in March 2004, in orchards located in the region of Araraquara – SP, hence the problematization of this work, in verifying the behavior of the disease after 16 years of its dissemination. The symptoms observed were associated with two species of bacteria, *Candidatus liberibacter* asiaticus and *C. liberibacter* americanus, (COLETTA-FILHO et al., 2004; TEIXEIRA et al., 2005), transmitted naturally in the field by Asian citrus psyllid, *Diaphorina citri* Kuwayama (LOPES et al., 2009).

A *D. citri* and stood out due to its rapid dissemination in orchards (FERNANDES, 2004) and wide range of hosts of the genus *Citrus* and related, with emphasis on *Murraya* spp. (LOPES et al., 2006). When the infective psyllid migrates from one orchard to another, the tendency is to concentrate on plants close to the peripheral part, causing the so-called edge effect, a hallmark of HLB (BASSANEZI et al., 2005; GOTTWALD et al., 2008) .

For HLB management, inspection is the most important tool, which aims to identify diseased plants to eradicate the inoculum source. In São Paulo, inspections are commonly carried out so that inspectors walk alongside plants and on inspection platforms attached and pulled by tractors. The main methods for combating the disease are the use of healthy seedlings, elimination of the inoculum by eradicating diseased plants, and control of the insect vector. For control to be efficient, there is a need for periodic inspections, spaced no more than 4 months apart, so the next step is to

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detection and immediate elimination of all plants identified with symptoms (BELASQUE JUNIOR et al., 2010).

According to Bové (2006) and Gottwald et al. (2007), knowledge of the proportion of plants that show symptoms and those that are asymptomatic is very important and must be taken into account when deciding to eliminate orchards with a high rate of infestation. According to what was proposed, the objective of the present work was to evaluate the incidence and behavior of HLB in the region of Araraquara, São Paulo.

2 BIBLIOGRAPHIC REVIEW

The data was obtained from analyzes by Fundecitrus (2020), demonstrating the following information:

Greening

Huanglongbing (HLB), also known as greening, is a devastating disease that attacks citrus farming and is considered one of the biggest threats to citrus farming worldwide (BOVÉ, 2006).

According to Coletta Filho and Carlos (2010), when contaminating the plant, the bacteria lodges itself inside the phloem vessels, affecting metabolism and causing the following symptoms: yellowing of the plant's branches; asymmetrical chlorosis on the leaf blade; deformation in fruits and aborted seeds; drought in the tips, loss of leaves and fruit fall.

As there is no cure to date, control of the disease consists of eradicating plants contaminated at the root, planting healthy seedlings and controlling the insect vector (BELASQUE JUNIOR et al., 2010).

To diagnose the disease, there are two methods that can be used, the Polymerase Chain Reaction (PCR) analysis described by Innis et al. (2009) and the visual inspection method carried out by pest pesters. The second was used to define Fundecitrus reports. However, these two methods have the disadvantages of high cost for PCR analysis, which makes their application as an effective method for control unfeasible.

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of the spread of the disease, and the visual inspection method only allows the detection of symptomatic plants.

It was observed that the average incidence of orange trees with greening symptoms in the citrus belt of São Paulo and Triângulo/Southwest of Minas Gerais is 20.87%. This percentage corresponds to approximately 41.3 million trees. Where the current index is 9.7% higher than that of 2019, estimated at 19.02%. The margin of error is plus or minus 1.29 percentage points (which represents 6% of the average incidence), with 95% confidence. (FUNDECITRUS, 2020)

Regarding the seriousness and incidence of greening disease, it was described that of the symptomatic trees, 37.9% have less than 25% of the crown affected by symptoms (level 1); 23.7% with symptoms between 26% and 50% (level 2); 17.5% with symptoms between 51% and 75% (level 3); and 20.9% with symptoms in more than 75% (level 4). The incidence of trees at levels 1 and 2, that is, with up to half of the crown affected by symptoms, rose from 11.7% in 2019 to 12.9% in 2020, while the incidence of trees with more than half of the cup with symptoms (levels 3 and 4) increased from 7.3% in 2019 to 8.0% in 2020. Regarding the incidence in the region under study, the following was observed:

> The regions with the highest incidences continue to be Brotas (60.46%), Limeira (53.18%), Porto Ferreira (33.67%) and Duartina (30.81%). Of these four regions, the incidence of greening increased in Brotas (+9.7%), Limeira (+10.1%) and Porto Ferreira (+26.2%) and decreased in Duartina (-5.0%). In an intermediate range of incidence are the regions of Avaré (16.77%), Altinópolis (15.73%) and Matão (14.47%). Of these three regions, the incidence continues to increase in Avaré (+55.7%) and Altinópolis (+28.9%), while in Matão it follows a downward trend (- 16.3%). The regions with the lowest incidences are Bebedouro (8.92%), São José do Rio Preto (3.50%), Itapetininga (1.63%), Votuporanga (0.08%) and Triângulo Mineiro (0.08%) . Of these regions, Bebedouro follows an increasing trend (+9.4%), while in the others the incidence remained within the incidence range of the last five years (FUNDECITRUS, 2020, p.12).

It is noted that Matão, which encompasses the Araraquara region, is on a downward trend, 16.3% over 2019. It is observed that the regions of Bebedouro and São José do Rio Preto have the lowest incidence rate. It is noteworthy that these regions are on the outskirts of Araraquara. The observation now is the incidence on the age of the plants investigated and the incidence of the disease in these plants. The report contains the following notes:

> Regarding age groups, the highest incidence was observed in orchards over 10 years old (28.59%), followed by orchards from 6 to 10 years old (20.35%), from 3 to 5 years old (11.36%) and from 0 to 2 years (1.69%). In orchards 6 to 10 years old and over 10 years old, the incidence increased for the fourth consecutive year. In the age range of 3 to 5 years, the incidence increased again after falling in 2019 (from 9.09% in 2018 to 6.50% in 2019 and to 11.36% in 2020). In the 0 to 2 year age group, the incidence fell again after

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> increased in 2019 (from 1.13% in 2018 to 2.46% in 2019 and 1.69% in 2020), but this value is within the range of incidence values over the last five years. These results indicate good rigor in the control of greening in orchards up to 3 years (psyllid control and elimination of diseased plants) and less rigor in the elimination of diseased plants in adult orchards (over 5 years). (FUNDECITRUS, 2020 p. 12)

What can be said is that despite the fluctuation in the incidence in younger plants, what remains constant is that older plants have higher incidences of infections.

Regarding the analysis of the size of properties and the percentage of disease, it appears that small properties are those that suffer most from greening, where the report describes the following situation:

> The smaller the property, the greater the incidence of plants with greening symptoms. On properties with up to 10 thousand plants (average size estimated at up to 21 hectares), the incidence reduced from 47.49% in 2019 to 44.07% in 2020 (- 7.2%). In properties between 10.1 thousand and 100 thousand plants (average size estimated at 21.1 to 210 hectares), the incidence (30.83%) was similar to that in 2019 (31.10%), while in properties above 100 thousand plants, the incidence increased compared to 2019. On properties with 100.1 thousand to 200 thousand plants (average size estimated at 210.1 to 420 hectares), the incidence increased from 16.17%, in 2019, to 18. 93%, in 2020 (+17.1%). On properties with more than 200 thousand plants (estimated average size greater than 420 hectares), the incidence increased from 10.23% in 2019 to 12.89% in 2020 (+26.0%) (FUNDECITRUS, 2020 p .13)

Finally, the analyzes promoted by Fundecitrus (2020) point out the incidence in relation to the plots analyzed on the edges of the orchards. In this way, we obtained the following numbers: Regarding the citrus belt, it was observed in the Fundecitrus study (2020), that 76% of the plants investigated were on the edges of the properties, thus located 100m from the borders of these properties. In the plots of the other plants (24%) investigated, they presented an average incidence of around 23.42%, lower than those on the edges.

3 METHODOLOGY

Due to the pandemic caused by Covid-19, the survey was carried out with the Citriculture Defense Fund of Araraquara, SP, Fundecitrus and was limited to data from 2020. In this sense, samples of 2.5% of the total were analyzed of existing plots of the main orange varieties (Hamlin, Westin, Rubi, Valencia Americana, Seleta, Pineapple,

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Pera Rio, Valencia, Natal and Valencia Folha Murcha), in a total of 1,023 plots, with varieties that make up 97% of the total trees in the park. One result of the smaller sample size is that the statistical possibility of error in the average incidence of HLB increased from the 4% established for previous years (2,200 samples) to 6% in 2020 (1,000 samples). It is also worth highlighting that the error is greater for the averages within the strata.

The method for selecting plots was carried out by draw, using the proportional stratified sampling technique, with samples being obtained from the 12 regions, assisted by Fundecitrus, being organized as follows: four property size groups and four age groups. The formation of these 12 regions is scaled by these regions: Triângulo Mineiro, Bebedouro, Altinópolis, Votuporanga, São José do Rio Preto, Matão, Duartina, Brotas, Porto Ferreira, Limeira, Avaré and Itapetininga, as shown in figure (1) below :

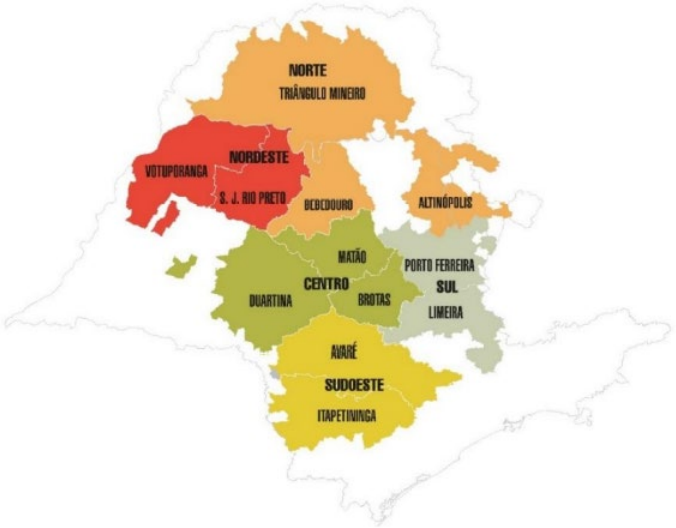

Figure 1 – Division of the citrus belt into 5 sectors and 12 regions.
Source: Tree inventory 2020 (Fundecitrus).

Property sizes are divided according to the number of trees: up to 10 thousand trees; from 10.1 thousand to 100 thousand trees; from 100.1 thousand to 200 thousand trees and above 200 thousand trees. It is also targeted on age: under 3 years; from 3 to 5 years; from 6 to 10 years and over 10 years.

The evaluation was carried out as follows: in each selected coupon, 11 trees were evaluated. In 2020, the plants from the 16th to 26th position of the 13th line of the field were inspected. About 82% of the trees inspected were 20 to 80 meters away from the edge; 16.53% less than 20 meters and 1.79% above 80 meters (Graph 1). The position of these trees that

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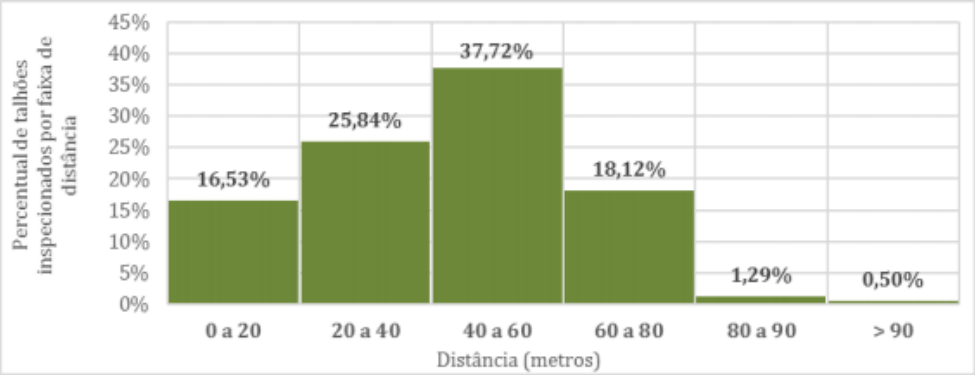
were inspected is ideal for estimating the incidence of greening, due to the transition region between the edge of the plot and its interior, which avoids over or underestimating the incidence of the disease (GRAVENA, 2005).



Source: Tree inventory 2020 (Fundecitrus).

In the analyzes carried out with the observation of Fundecitrus (2020), an identification was constructed that estimates the severity of disease symptoms in leaves and/or fruits. For greening and CVC, a grade from one to four was assigned, equivalent to: up to 25% of the canopy with symptoms (level 1); from 26% to 50% (level 2); from 51% to 75% (level 3) and from 76% to 100% (level 4). The surveys and audit were carried out in the first half of 2020.

4 RESULTS

When comparing the incidences of greening in the regions presented in the Fundecitrus report (2020), there are differences arising from several factors, such as the place where the epidemic began in the citrus belt, the psyllid population and the incidence of greening in the previous year . Regarding the last two items mentioned, there is a direct relationship with variables related to climate variability for the reproduction of the psyllid and multiplication of the bacteria in diseased plants and also with factors that facilitate or hinder the management of the disease, such as the profile of citrus properties (size), the density of citrus properties in the region (proximity between citrus orchards) and the rigor in the application of internal and external control measures by citrus growers. It is also worth mentioning that the rates of eradication of affected orchards, of new plantings and replantings and of the formation of new orchards also affect the incidence of greening in the region.

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The first reports of greening disease were evidenced in the regions of Matão and Porto Ferreira, spreading through psyllids like a wave throughout the other regions of the citrus belt. The longer period of occurrence of greening in the central regions has led to higher incidences, with a gradual reduction as one moves further towards the north and west (Triângulo Mineiro and Votuporanga) and south (Itapetininga), located more than 200 km from the center of the citrus park.

There is a relationship with the incidence of the disease and psyllid populations, as the regions with the highest incidences of the disease in 2020 are also those with the highest psyllid populations observed by the Fundecitrus Phytosanitary Alert system in previous years (2018 and 2019). While in regions with a lower incidence of the disease, such as Votuporanga, São José do Rio Preto, Triângulo Mineiro, Itapetininga and Bebedouro, the population of psyllids captured by 14 traps per fortnight (paq) was, respectively, 0.021, 0.049, 0.021 and 0.097 paq. In regions with the highest incidence of the disease, such as Brotas, Limeira, Porto Ferreira and Duartina, the psyllid population was 0.243, 0.389, 0.227 and 0.242 paq, respectively. In regions with intermediate incidences, such as Matão, Altinópolis and Avaré, the psyllid population was 0.243, 0.188 and 0.172 paq, respectively (FUNDECITRUS, 2020).

The climatic variation between the regions of the citrus park is very large, especially in the autumn and winter months. This situation, historically, demonstrates that there was a gradient in rainfall (which decreases) and temperature (which increases) as a result of moving from the Southwest sector, passing through the Center and South, and reaching the North/Northwest. During autumn and winter, the cold/rainy fronts coming from the South Pole lose strength as they advance to the north of São Paulo and the Triângulo Mineiro region.

From this perspective, it rains more in the Southwest sector, the rain is better distributed over time, and temperatures are lower than in other regions. Further north, with the lack of water combined with higher temperatures, plants suffer from water deficits, which in turn affect the shoots, the psyllid and the HLB bacteria.

Thus, in the Southwest sector, due to the greater amount of rain, sprouts tend to be more frequent. However, low temperatures reduce the psyllid's reproduction rate, which reaches smaller populations in the colder months, thus reducing the spread of HLB.

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The Itapetininga region has the lowest psyllid capture rates recorded in the Phytosanitary Alert system, considering the average for the years 2018 and 2019. The Avaré region also had low psyllid captures (average of 0.082 paq) until 2018, however, as of 2019, the psyllid population increased significantly (0.253 paq).

This helps to explain the historically lower incidences in the regions of Itapetininga and Avaré, and the increase in Avaré this past year. In the North and Northwest sectors, due to the water deficit and higher temperatures, sprouts are less frequent than in the South/Southwest, and the peaks are better defined. This leads to a reduction in the psyllid population and lower concentrations of the bacteria in the shoots of diseased plants.

The lower the concentration of bacteria in the shoots, the lower the rate of acquisition by the psyllid and, consequently, the rate of spread of the disease. This helps to explain the historically lower incidences in the regions of Triângulo Mineiro, Votuporanga, São José do Rio Preto and Bebedouro, which also have lower capture rates of psyllids registered in the Phytosanitary Alert system, considering the average for the years 2018 and 2019. In Bebedouro, the capture of 15 psyllids was even lower (0.035 paq) until 2017, when it began to gradually increase. The exception is the Altinópolis region, whose psyllid population observed in the last two years has been intermediate, which explains the increased incidence of greening.

FINAL CONSIDERATIONS

The presence of the transmitting vector on the property, the psyllid, greatly increases the probability of occurrence of both symptoms and the disease itself, and is therefore an important indicator that can be used in practice. On the other hand, the occurrence is greater in crops with smaller size properties and number of plants. The probabilistic sampling survey proved to be a useful tool for knowing the incidence of a disease in plants, particularly in citrus, even in this last year, where there were major restrictions that affected the field research carried out by Fundecitrus (2020). However, good quality results were obtained, with good precision and in a short period of time.

The sampling process proves to be especially efficient at the beginning of the disease's occurrence, when there is not yet much information or resources available to inspect all the plants, but a quick response regarding the disease is needed.

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severity of the situation. Obviously, when moving on to the phase of treating or eradicating plants, one cannot do without a complete scan, that is, inspection of all of them, as, in this case, it is necessary to find all the diseased plants and not estimate their number.

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