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Association of Temperature, Relative Humidity, and Shoot Size Scale with the Number of Eggs and Nymphal Stages of *Diaphorina citri* (Hemiptera: Liviidae)

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Abstract

The objective of the study was to determine the influence of temperature, relative humidity, and shoot size of Valencia orange trees *Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae) on the abundance of eggs and nymphal stages of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). The experiment was established on 3.18 hectares cultivated with Valencia orange. The number of eggs, nymphs, temperature, relative humidity, and scale of the size of the shoot were recorded from January to July and from September to November 2020. The association of these variables was determined by multiple correspondence analyses. The conservation of the same number of individuals between consecutive samples and the increase in the number of eggs and nymphs was associated with temperature (17˚C - 23˚C), relative humidity (75% - 78%) and the availability of shoots from V1 to VS in March, April, June, and July. The largest number of N1 and N2 nymphs was recorded in January, February, May, and October. The highest population of eggs and nymphs N3 and N5 occurred in September. In November, there was a reduction in eggs and nymphs. Meanwhile, the nymph N4 was presented independently of the variables analyzed.

Keywords

Citrus sinensis, HLB, Monitoring, Psyllid, Shoots

1. Introduction

In Mexico, the state of Tamaulipas is the second largest producer of Valencia oranges *Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae) [1] [2]. In this state, to December 2015, the bacterium *Candidatus* Liberibacter asiaticus (*C*Las) Jagoueix (Rhizobiales: Rhizobiaceae) was reported in Valencia orange trees [3] [4]. The bacterium *C*Las causes the disease known as Huanglongbing or HLB [5]. HLB causes the death of trees by plugging the phloem with starch [6] [7]. The bacteria can be acquired and spread by nymphs and adults of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). In the case of nymphs, their biological development is affected by temperature and relative humidity [8] [9]. For example, the full development of nymphs is prolonged by a temperature below 10˚C or above 33˚C [10]. Likewise, nymphs can survive in extreme environments ranging from −7˚C to 45˚C in arid climates and humid subtropical areas [11] [12]. Relative humidity affects insect biology because nymphs are intolerant to relative humidity greater than 87% [13]. In the field, humidity above 80% increases the mortality of nymphs due to the development of fungal epizootics [14]. Under controlled conditions, the behavior is reversed since the survival of nymphs increases with relative humidity above 97% [15] [16].

The occurrence of eggs and nymphs of *D. citri* is related to the developing shoots of citrus trees. Growing vegetative shoots provides conditions for egg-laying and incubation and the emergence and development of *D. citri* nymphs [17]-[19]. For example, the length of shoots influences the abundance of eggs and nymphs in a laboratory and at the field level. In the 1 mm shoots, the beginning of oviposition occurs; the 3 mm shoots are key to the biological development of the nymphs [20], and in the 25 mm, 100 mm, and 150 mm shoots, densities of 25 to 100 nymphs are recorded [21]. Tamaulipas has temperatures below 12˚C and above 26˚C, and percentages of relative humidity between 0.2% and 73.4% [22], favorable conditions for the development of *D. citri*. Likewise, it is known that *C. sinensis* shoots are produced from January to July and from October to December [23]. However, in Tamaulipas, the association between climatic conditions and the production of vegetative shoots in the abundance of eggs and nymphs of *D. citri* is unknown. Therefore, the objective of this study was to determine the influence of temperature, relative humidity, and the scale of shoot size on the abundance of eggs and nymphal stages of *D. citri* in a commercial garden grown with Valencia orange, located in the citrus zone of Tamaulipas.

2. Materials and Methods

2.1. Study Area

From January to July and from September to November 2020, the study was carried out in the orchard "Luz del Campesino 2" located in the municipality of Güémez, Tamaulipas, Mexico (**Figure 1**). The municipality of Güémez is located between the parallels 24˚06' and 23˚41' north latitude, the meridians 99˚30' and 98˚45' west longitude, and at an altitude between 200 and 2800 meters above sea level [22]. The climatic condition of the municipality varies, given that there are temperatures below 12˚C and above 26˚C [22]. Precipitation is between 600 and

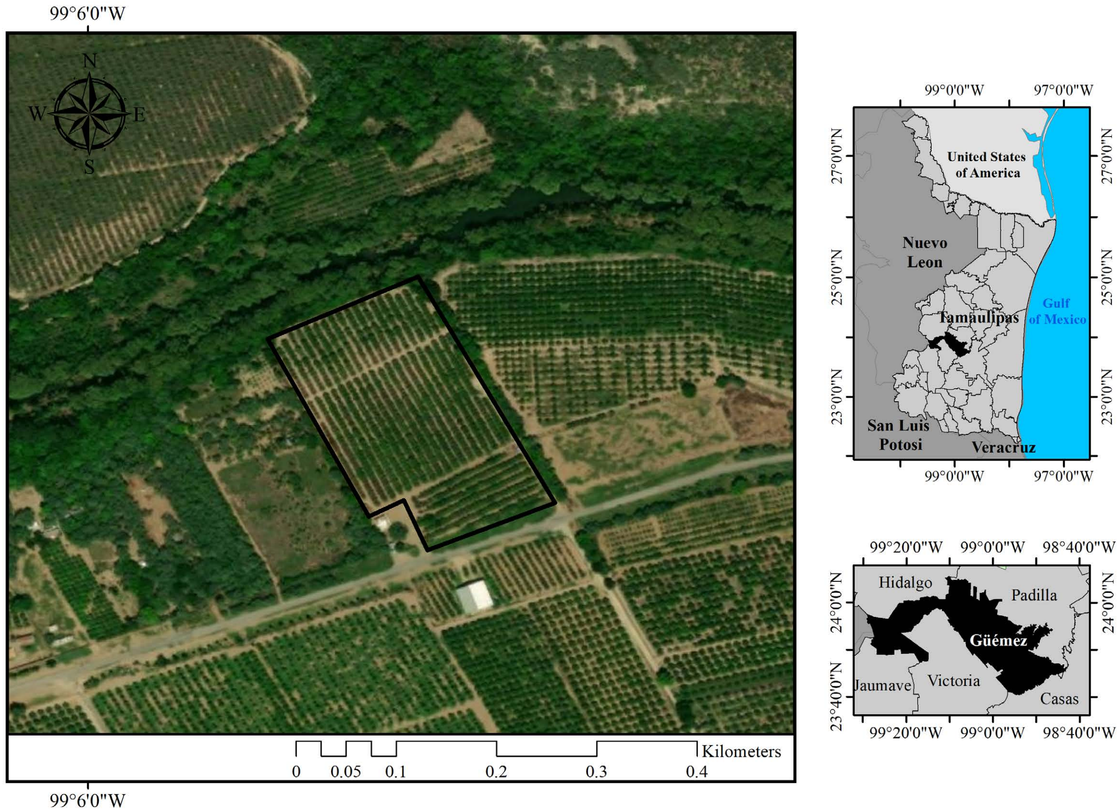

Figure 1. Geographical location of the orchard "Luz del Campesino 2" in the municipality of Güémez, Tamaulipas, Mexico.

1100 mm per year, with a percentage of relative humidity from 0.2% to 73.4% [22]. The orchard "Luz del Campesino 2" is composed of 3.18 hectares planted with Valencia orange *C. sinensis*, with agricultural work ranging from the application of insecticides, fertilization, and a micro-spray irrigation system.

2.2. Egg and Nymph Monitoring

The sampling of the eggs and the nymphal stages was carried out based on the method proposed by the National Service of Health, Safety and Agri-food Quality (SENASICA in Spanish) [2]. This sampling consisted of carrying out a systematic sampling in the form of a simple "T" in 30 trees to generate information on the number of insects on the edge of and inside of the orchard. Then, the number of eggs and each nymphal stage (N1-N5) was counted in a shoot of 30 citrus trees and samplings were held every Monday, Wednesday, and Friday from January to July and from September to November 2020 (budding period).

2.3. Recording of Temperature and Relative Humidity

The temperature and relative humidity were recorded through an automatic climate data logger (HOBO Datalogger) with the HOBO mobile® application for iOS version 2.2 (2019). The data logger was placed at the center of the simple "T" at the orange orchard throughout the development of this study.

2.4. Record of the Size of the Shoot

The scale of the shoot size was delimited based on the criteria proposed by SENASICA in the operational manual of the campaign against regulatory pests of citrus fruits [2]. This manual establishes that the classification of the vegetative shoot is based on a scale of V1 to V7 according to its budding phase (bud, bud growth), shoot size in centimeters (1 - 20), and change time in days (7 - 60). The V6 and V7 scale, however, have maturation characteristics of the young branches. Therefore, the shoot size scale record in the present study consisted of the V1 - V5 scale. At the same time, the VS scale was integrated for the shoots that withered through the sampling period (**Figure 2**).

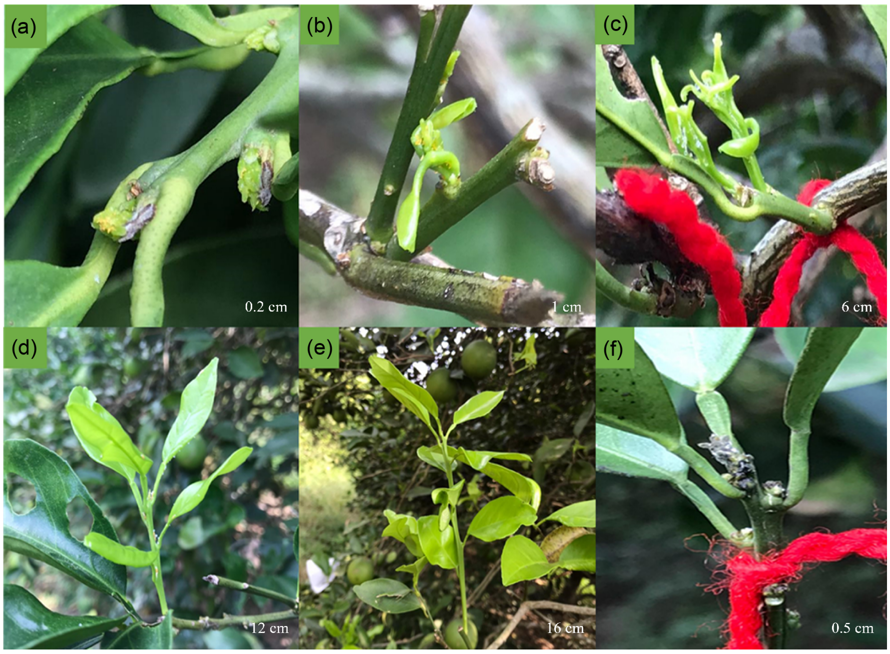

Figure 2. Characteristics of the scale values of Valencia orange in the orchard "Luz del Campesino 2" of the municipality of Güémez, Tamaulipas, Mexico. Where: a = V1, b = V2, c = V3, d = V4, e = V5 and f = VS.

2.5. Statistical Analysis

The association of egg number and nymph population relative to sampling month, sampling week, shoot size scale, temperature, relative humidity, egg number, and the number of each nymphal stage was analyzed with Multiple Correspondence Analysis (MCA) [23]. The sampling month was divided into 10 categories (January, February, March, April, May, June, July, September, October and November), according to the budding period of Valencia orange trees; the sampling week into four categories (S1, S2, S3 and S4), as a function of the number of weeks during each sampling month; the shoot size scale into six categories (V1, V2, V3, V4, V5 and VS), based on the scale proposed by SENASICA, and the shoot that dried out over time; the temperature (17˚C, 21 C, 23˚C, 25˚C and 27˚C) and relative humidity (72%, 75%, 78%, 84% and 87%) in five categories, according to seasonal averages during the study period (typical conditions except relative humidity); the number of eggs in one categories category (Eg) and the number of nymphs in five categories (N1, N2, N3, N4 and N5), in function of the development stages of *D. citri*. In turn, an average of 50 individuals accounted for the increase (IN), reduction (REDUC), and conservation of the same number of individuals through consecutive sampling (CONS). The analysis combined more than two categorical variables, so the value of X^2 is not valid. The identification of the association between the categories that compose it was made based on a two-dimensional graph that shows the association between the categories, where the degree of association between categories of different variables is reduced by increasing the distance between them [24] [25]. The center of origin of the graph (0.0) corresponds to the average abundance of the species for each of the categories. At the same time, using the contribution table X^2 generated by the MCA, the percentage of variation of the variables to the canonical space was calculated, which was obtained from the division of the total (X^2) of the variable, with the total (X^2) of all the variables multiplied by 100.

3. Results

The frequency of the number of eggs and the five nymphal stages of *D. citri* differed throughout the sampling period. The greater variation in the frequency of the number of eggs and nymphs (N1 - N5) in the canonical space of the first two dimensions of the multiple correspondence analysis was explained by the sampling month (27.48%) and the scale of the shoot size (20.00%). Followed by temperature (16.43%), relative humidity (16.38%), species category (8.44%), sampling week (6.70%), and conservation, increase and reduction of egg abundance and each of the nymphal stages (4.53%). Stage nymphs N1 and N2 tended to be found more frequently during January, February, May, and October at temperatures between 17˚C to 23˚C, relative humidity between 75% to 78%, and shoots on size scales from V1 to V5. In contrast, in March, April, June and July, the frequency of eggs and nymphs was preserved and increased (+50 eggs and nymphs) during the second week of sampling at a temperature between 25˚C and 27˚C, with a relative humidity of 72% and growing shoots (V1 - V5) or dry (VS). On the other hand, in September, the frequency of eggs and nymphs in stages N3 and N5 was more frequent when the temperature was at 21˚C, relative humidity at 84%, and shoots were maintained on V2 scales. Finally, in November, the frequency of eggs and nymphs was reduced between samplings (−50 eggs and nymphs) during the third and fourth weeks when relative humidity was found at 87%, and shoots were maintained on V3 scales. The frequency of nymphs in stages N4 was like the other categories of the variables analyzed because the category of nymph N4 had an inclination and diameter close to the average (**Figure 3**).

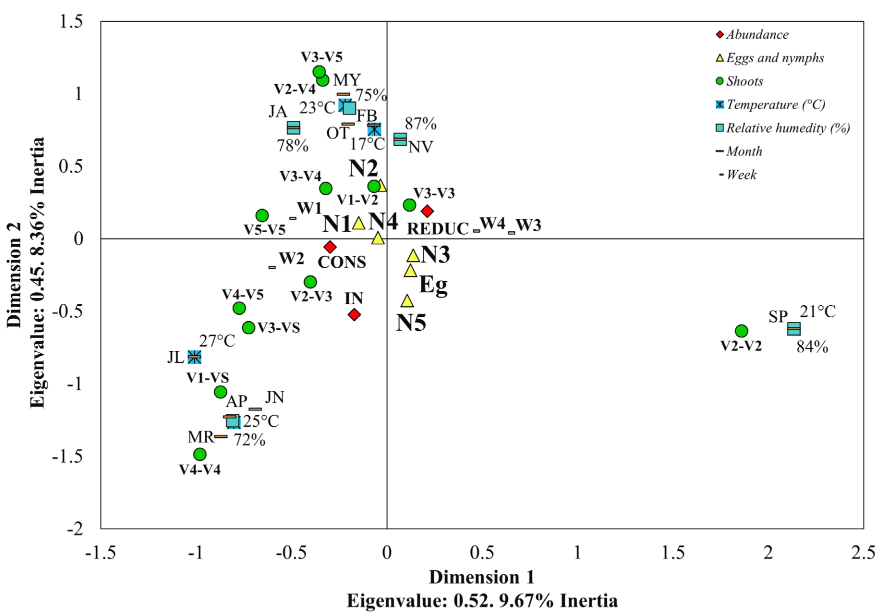

Figure 3. Multiple correspondence analysis showing the ordering of the abundance of eggs (Eg) and nymphs (N1 - N5) with the variables of temperature (˚C), relative humidity (%), scale of the size of the shoot (V1 - VS), and sampling time (month and week). Where: JA = January, FB = February, MR = March, AP = April, MY = May, JN = June, JL = July, SP = September, OT = October and NV = November; W1 = Week 1 (first week of sampling at the beginning of each month), W2 = Week 2 (second week of sampling at the beginning of each month), W3 = Week 3 (third week of sampling at the beginning of each month) and W4 = Week 4 (fourth week of sampling at the beginning of each month); V1 = shoot scale 1, V2 = shoot scale 2, V3 = shoot scale 3, V4 = shoot scale 4, V5 = shoot scale 5 and VS = dry shoot scale; Eg = Egg, N1 = Nymph 1, N2 = Nymph 2, N3 = Nymph 3, N4 = Nymph 4 and N5 = Nymph 5; CONS = Conservation of the same number of eggs and nymphs (N1 - N5), IN = Increase in eggs and nymphs (N1 - N5) and REDUC = Reduction of eggs and nymphs (N1 - N5).

4. Discussion

The environmental conditions of March, April, June, and July favored the laying and incubation of eggs, as well as the emergence and development of all nymphal sizes. In contrast, conditions in January, February, May, and October only favored N1 and N2 nymphs. In September, environmental conditions only favored the laying and incubation of eggs and the nymphs of stages N3 and N5. The results presented in the present study are the first records of the effect of environmental factors on egg oviposition and incubation and *D. citri* nymph sizes in the Valencia orange crop in Tamaulipas, Mexico. In this regard, in Mexico, there are only reports of temporary variations of eggs and nymphs in citrus shoots. For example, in the citrus zone of the State of Veracruz, the highest number of eggs occurs in May (41 eggs/shoot) and March (29 eggs/shoot), and the largest number of nymphs occurred in March (74 nymphs/shoot) and June (43 nymphs/shoot) [26]. In other countries such as Pakistan, the greatest abundance of nymphs was recorded in April and September, with a population average of 12.75 and 11.8 nymphs per Valencia orange shoot [27]. In India, the nymph population is highest in March and September, with a population average of 15.2 and 15.7 nymphs per Valencia orange shoot [28]. In contrast, in the United States of America, in grapefruit cultivation, the egg and nymph populations were higher in March, May, and June, with an average of 26.5 eggs and 16.8 nymphs per shoot [29].

In the present study, at the field level, temperature and relative humidity had a greater effect in regulating the abundance of eggs and nymphs of *D. citri* of different sizes. The result improves the understanding of the degree of participation of both factors in the biological cycle of *D. citri* in the field since it was currently only known that temperature regulates the number of generations of *D. citri* per year and that oviposition is affected only when the relative humidity is less than 40% [21] [30]. However, the effect of relative humidity in the present study contradicts previous reports since oviposition was adversely affected by the relative humidity recorded above 80%. Nevertheless, when the relative humidity fluctuates, a negative effect occurs in the oviposition and development of *D. citri* nymphs [31] [32]. However, *D. citri* can adapt to variations in temperature and relative humidity [13] [14]. Similarly, other authors have pointed out that in the face of changes in temperature and humidity, nymphs may adapt to the rate of water loss that modulate their development and temperature [15] [33] [34]. Because of this, nymphs can survive a wide range of extreme temperatures ranging from −7˚C to 45˚C in arid climates and humid subtropical areas [11] [12]. In the present study, this behavior was observed in N4 nymphs because its occurrence was not associated with the variables analyzed.

The presence of insects in a specific space can occur due to a combination of abiotic and biotic factors, so that, in addition to humidity and temperature, the developing shoots of the orange trees constitute a fundamental factor in the presence of *D. citri* in the environment [16] [20] [35] [36]. The results obtained in the present study coincide with those authors since, in the Valencia orange trees, an increase in the number of eggs and nymphs was observed when the host plant had shoots at different scales of development. In field and laboratory studies, the oviposition and development of *D. citri* nymphs are induced by the budding period and the availability of growing shoots [37]-[40]. Therefore, in natural conditions there is the possibility of a population increase due to the availability of shoots both in orange and in the of lemon trees [14] [41] [42]. So that, the period of continuous budding of the lemon trees favors the oviposition and nymphal development of *D. citri* [43]. However, the selection of the shoot involves factors such as the location of the host, which is determined by visual and olfactory signals and nutritional condition of the trees [44]-[46]. On the other hand, younger shoots are the most conducive to oviposition and development of nymphs based on their chemical composition, lignified tissues, and length [17] [18] [39]. For example, shoots with a length of 3 to 50 mm (V1 - V3) have a higher abundance of oviposition compared to shoots greater than 50 mm (V4 - V5), where oviposition is practically absent [42] [47]. However, shoots with a length of 25 to 150 mm (V3 - V5) have densities of 25 to 100 individuals *D. citri* per shoot [21]. In this sense, the characteristics of vegetative shoots provide the insect with conditions for its oviposition and the development of nymphs [17] [20] [48].

On the other hand, at the field level, studies focused on the association of biotic and abiotic factors with the population size of eggs and nymphs of *D. citri* are scarce because the eggs are very small and the count is complex, in the same way, the number and stage of nymphs is difficult to count [49]. In the present research, data record of eggs, the classification of nymphs, the scale of the shoot size, the time of registration of the data and the ignorance of the timing of chemical application and irrigation schedules played an important role in generating this information. This is because during the sampling period, residues of chemical and irrigation applications were detected, which were not contemplated in the study. Therefore, future research should establish an experiment where it is possible to cut the shoots to count eggs and nymphs with the help of a stereoscopic microscope in the laboratory. It is also recommended to generate a new scale of the sizes of shoots contemplating the number and size of the leaf's information since, with the current scale, it is complicated to classify the shoots in the field. Also, to rule out other factors, it is necessary to include other variables. For example, the record of parasitism and predation, the frequency of the different scales of shoot size in a single branch, the morphological characteristics for the classification of the shoot, the average number of shoots that are generated per tree, the carrying capacity of eggs and nymphs that the shoot can support, and the timing of chemical and irrigation applications in the region. Finally, the occurrence of eggs and nymphs in the face of abiotic and biotic factors detected in the study area is basic information for designing management plans against *D. citri*. In this sense, it is suggested that the shoot scales V1, V2 and V3 act as objectives in the management plan to suppress the development of the adult and consequently the spread of HLB.

5. Conclusion

The conservation and increased frequency of eggs and nymphs were associated with the sampling period of March, April, June and July, a temperature of 17˚C - 23˚C, a relative humidity between 75% and 78%, and the availability of orange shoots on scales from V1 to VS, with a majority between V2 and V3. In contrast, a reduction in egg and nymph abundance was observed during November. On the other hand, in the period comprising January, February, May and October, nymphs in stages N1 and N2 were more frequent, while in September the presence of eggs and nymphs in stages N3 and N5 were more abundant. Likewise, the N4 nymph is more frequently present during any period, regardless of the variables analyzed. Then, the frequency of the number of eggs and of each of the nymphs' stages was associated with the variability of abiotic and biotic factors because of the time of study.

Authors Contributions

Jesús Armando Vargas-Tovar established the research objectives, contributed to field sampling, data analysis and writing of the article; Crystian Sadiel Venegas-Barrera collaborated in the writing and analysis of the data; Martha Olivia Lázaro-Dzul collaborated in the revision and writing of the article; Vidal Zavala-Zapata collaborated in the field sampling; Ausencio Azuara-Domínguez collaborated in the establishment on the study area, helped in the general correction of the article and was in charge of advising the research together with the first author.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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