Article

Seasonal abundance of *Diaphorina citri* associated with the phenology of citrus crops

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Abstract

In the present research, the participation of the phenology of the Valencia orange (*Citrus sinensis* L. Osbeck) and Italian lemon (*Citrus limon* L. Burn f.) crops in the seasonal abundance of *Diaphorina citri* Kuwayama was determined. The study was carried out during the years 2015 to 2018 in three regional control areas (ARCOs), located in the municipalities of Padilla, El Barretal, El Carmen, Estación Santa Engracia, Güémez and Victoria, Tamaulipas. In these areas, the abundance of *D. citri* in each phenological phase of the orange crop (ARCO 1 and 2) and lemon (ARCO 3) was recorded. The Italian lemon crop produced shoots all year round. Meanwhile, the Valencia orange crop presented a phase of dormancy. In the four years, the seasonal abundance of *D. citri* behaved differently in the three ARCOs. The greatest abundance of the insect occurred in the phase of shoot production in trees. The result obtained in the present work could be used to define the period of application of the insecticides recommended for the control of *D. citri* in the ARCOs.

Keywords: *Diaphorina citri*, arco, HLB, shoots.

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Introduction

In Mexico, the first report of HLB or Huanglongbing occurred in July 2009 and by November 2015 the disease was recorded in 347 municipalities in 18 citrus-producing states in Mexico (Garza *et al*., 2017). The estimated economic and social impact of the presence of this disease, just three years after its arrival, was 1.7 million tonnes of fruit and 112.1 million lost wages (Díaz *et al*., 2014). To mitigate this, the phytosanitary campaign against HLB was designed and implemented. The activities carried out in the campaign against HLB were initially focused on the detection and search for symptoms caused by the causative agent of the disease, the bacterium *Candidatus* Liberibacter asiaticus Jagoueix (Rhizobiales: Rhizobiaceae).

Subsequently, they focused on monitoring the insect vector, *Diaphorina citri*, of the bacterium in order to know the population distribution of the pest insect in time and space (Flores *et al*., 2017), and measure the impact of the insecticides contemplated in the strategy against HLB in the regional populations of the insect. In Tamaulipas, *D. citri* was first recorded in 2003 (López-Arroyo, 2005). In 2014, the State Plant Health Committee of Tamaulipas (CESAVETAM, for its acronym in Spanish) established a monitoring program with yellow sticky traps under the scheme of regional control areas (ARCOs) in order to control the spread of the pest insect and bacterial disease in 33 272.12 ha of orange (*Citrus sinensis* L. Osbeck) (Sapindales: Rutaceae) and 3 339.31 ha of lemon (*Citrus limon* L. Burn f.) (Sapindales: Rutaceae) (SIAP, 2019), located in the municipalities of Padilla, El Barretal, El Carmen, Estación Santa Engracia, Güémez and Victoria (Mora *et al*., 2014).

Due to the economic importance of citrus production in these municipalities, it is necessary to analyze the seasonal abundance of *D. citri* in Valencia orange and Italian lemon trees in order to avoid the presence of HLB. Therefore, the objective of this research was to determine the seasonal abundance of *D. citri* associated with the phenology of the Valencia orange and Italian lemon crops.

Materials and methods

Study area

The experiment was established during the years 2015 to 2018 in three regional control areas (ARCOs) located in the municipality of Padilla, El Barretal, El Carmen, Estación Santa Engracia, Güémez and Victoria, Tamaulipas (Figure 1).

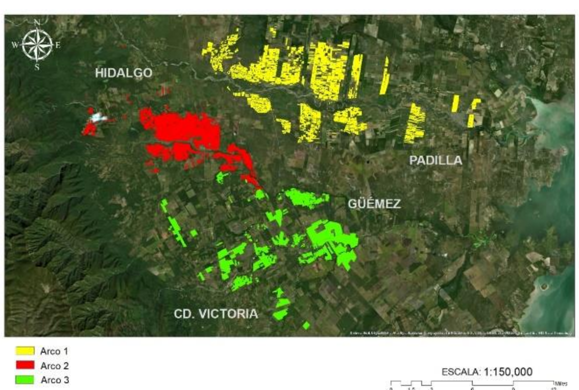

Figure 1. Geographical location of the regional control areas (ARCOs) in Tamaulipas, Mexico.

ARCO 1 was made up of 5 402.38 ha, 65 commercial orchards (Table 1) sown with Valencia orange trees. The average age of the trees was 15 years, the separation between the trees and lines was four and seven meters. The trees were watered with a furrow irrigation system.

Table 1. Area and geographical location of the orchards of Valencia orange *Citrus sinensis* (L.) Osbeck of ARCO 1 located in the municipality of Padilla and El Barretal.

Commercial orchard	Area (ha)	Latitude	Longitude
Ejido La Soledad 6	57	24.086535	-99.057106
Ejido Mártir de Chinameca 2	125.75	24.113646	-99.09361
Ejido Conrado Castillo 4	14.5	24.08759	-99.02356
Ejido José López Portillo 7	4	24.04135	-99.00219
Ejido La Soledad 2	57	24.118633	-99.070244
Ejido La Soledad 9	64	24.104939	-99.054146
Ejido San Patricio 2	132	24.060936	-99.079445
Orchard El Tejón 2	50	24.03793	-98.886696
Ejido Conrado Castillo 3	14.5	24.070562	-99.03035
Ejido José López Portillo 6	34	24.048689	-99.0219
Ejido La Soledad 4	57	24.132109	-99.067894
Ejido Conrado Castillo 10	15.39	24.056007	-99.03695
Ejido Conrado Castillo 5	14.5	24.077688	-99.02941
Ejido Plan de Ayala 3	192.83	24.043032	-99.09813
Ejido Plan de Ayala 1	192.84	24.032415	-99.10381
Ejido Guadalupe Victoria 1	41	24.063482	-99.12173
Ejido La Soledad 5	57	24.134998	-99.06753
Ejido Guadalupe Victoria 5	41	24.064547	-99.14512
Ejido San Patricio 3	132	24.058231	-99.07481
Ejido La Soledad 7	57	24.09412	-99.055435
Ejido Guadalupe Victoria 9	41	24.072016	-99.15731
Ejido Conrado Castillo 7	14.5	24.050945	-99.03912
Ejido Marte R. Gómez 7	72.95	24.09832	-99.03486
Ejido José López Portillo 4	34	24.041721	-99.00998
Ejido San Patricio 1	132	24.042892	-99.085311
Ejido José López Portillo 5	34	24.041246	-99.02064
Ejido Mártir de Chinameca 1	125.75	24.104736	-99.11817
Ejido Marte R. Gómez 6	72.95	24.08675	-99.0412
Ejido Guadalupe Victoria 8	41	24.065233	-99.145355
Ejido La Soledad 8	57	24.08344	-99.0645
Ejido La Concepción 2	83	24.106049	-99.05002
Orchard El Paraíso	100	24.08466	-99.08274

Commercial orchard	Area (ha)	Latitude	Longitude
Ejido Marte R. Gómez 5	72.95	24.082705	-99.04501
Ejido Marte R. Gómez 1	5	24.072016	-99.044785
Ejido La Concepción 1	83	24.09197	-99.04489
Ejido La Concepción 4	83	24.082035	-99.05381
Ejido Guadalupe Victoria 2	41	24.065823	-99.12232
Ejido Cruz y Cruz 1	382	24.09858	-99.18552
Ejido José López Portillo 2	34	24.0361	-99.0126
Ejido Conrado Castillo 8	14.5	24.050976	-99.01905
Ejido Guadalupe Victoria 3	41	24.067486	-99.131035
Ejido Guadalupe Victoria 6	41	24.054068	-99.13169
Ejido Guadalupe Victoria 4	41	24.06885	-99.13532
Ejido José López Portillo 1	34.13	24.035378	-99.01616
Ejido La Concepción 5	83	24.076841	-99.05645
Orchard Lote 47	45	24.057177	-98.88358
Ejido Guadalupe Victoria 10	41	24.070724	-99.15082
Ejido Nuevo San Juan 1	258	24.047245	-99.04309
Ejido Guadalupe Victoria 11	41.96	24.077404	-99.16936
Ejido Plan de Ayala 2	192.83	24.041084	-99.105446
Ejido La Soledad 3	57	24.127895	-99.07232
Ejido Marte R. Gómez 3	72.95	24.068739	-99.04935
Ejido Conrado Castillo 6	14.5	24.051632	-99.04219
Ejido Carmen Galindeño 1	681.7	24.094416	-99.141716
Ejido Conrado Castillo 1	14.5	24.075184	-99.02093
Ejido Conrado Castillo 9	14.5	24.056099	-99.02827
Orchard El Tejón 1	50	24.038193	-98.889534
Ejido Marte R. Gómez 2	72.95	24.06817	-99.0434
Ejido Marte R. Gómez 4	72.95	24.076044	-99.05218
Ejido Guadalupe Victoria 7	41	24.056377	-99.13623
Orchard El Lucero	100	24.096743	-98.98633
Ejido José López Portillo 3	34	24.041996	-99.01711
Ejido José Silva Sánchez 2	233	24.124647	-99.05361
Ejido José Silva Sánchez 1	233	24.08227	-99.07351
Ejido Conrado Castillo 2	14.5	24.06979	-99.02477

ARCO 2 was made up of 3 491.23 ha, 49 commercial orchards (Table 2) sown with Valencia orange. The average age of the trees was 15 years, the separation between the trees and rows was four and seven meters. The trees were watered with a furrow irrigation system.

Table 2. Area and geographical location of the orchards of Valencia orange *Citrus sinensis* (L.) Osbeck of ARCO 2 located in the El Carmen and Estación Santa Engracia.

Commercial orchard	Area (ha)	Latitude	Longitude
Ejido El Arco 8	22.65	23.99976	-99.25969
Ejido El Arco 1	22.65	24.009975	-99.256165
Ejido El Arco 4	22.65	24.011806	-99.26059
Ejido San Isidro 2	43	24.015835	-99.24684
Ejido El Arco 6	22.65	24.018017	-99.26326
Ejido El Arco 3	22.65	23.988935	-99.171196
Ejido El Porvenir 3	18.5	23.99402	-99.259415
Ejido El Porvenir 2	18	23.984842	-99.17778
Ejido El Arco 10	22.65	23.991343	-99.254845
Ejido San Isidro 1	43.28	24.008848	-99.24169
Ejido El Arco 9	22.65	24.020386	-99.25239
Ejido El Arco 7	22.65	24.008661	-99.248955
Ejido El Arco 2	22.65	24.012655	-99.24023
Ejido Guillermo Zúñiga 6	74.14	24.044983	-99.20357
Ejido El Arco 5	22.65	24.018524	-99.25535
Ejido Guillermo Zúñiga 3	74.14	24.019312	-99.19423
Ejido Vicente Guerrero 1	61	24.035128	-99.21106
Ejido Emiliano Zapata 3	90.51	24.035948	-99.23369
Ejido Guillermo Zúñiga 7	74.14	24.033747	-99.18054
Ejido Emiliano Zapata 1	90.51	24.037302	-99.22089
Ejido Emiliano Zapata 4	90.51	24.048454	-99.19514
Ejido Benito Juárez 3	94	24.04681	-99.220184
Ejido Guillermo Zúñiga 8	74.14	24.02302	-99.24403
Ejido Balconcitos 4	27.92	24.016384	-99.216675
Ejido San José de Santa Engracia 1	728.11	24.024101	-99.236465
Ejido Vicente Guerrero 2	61	24.044575	-99.211586
Ejido El Porvenir 1	18.5	23.984312	-99.181885
Ejido Vicente Guerrero 3	61	24.041195	-99.21406
Ejido La Diana 1	374.42	23.995256	-99.14137
Ejido Guillermo Zúñiga 5	74.14	24.033224	-99.19679
Ejido Benito Juárez 1	94	24.049835	-99.23015
Ejido Guillermo Zúñiga 9	74.14	24.050026	-99.20151
Ejido Guillermo Zúñiga 1	85.99	24.017195	-99.1816
Ejido Guillermo Zúñiga 2	74.18	24.025429	-99.1884
Ejido Balconcitos 6	27.92	24.018547	-99.2217

Commercial orchard	Area (ha)	Latitude	Longitude
Ejido Balconcitos 3	27.92	24.015446	-99.229996
Orchard las Enramadas	50	24.023846	-99.21707
Ejido Benito Juárez 2	94	24.042488	-99.244865
Ejido La Rosita 1	43.07	24.0049	-99.2263
Ejido La Rosita 3	43.07	24.008351	-99.22203
Ejido Balconcitos 1	27.92	24.011097	-99.20382
Ejido Guillermo Zúñiga 4	74.14	24.027617	-99.198105
Ejido Balconcitos 5	27.92	24.016468	-99.23209
Ejido Balconcitos 2	27.92	24.013247	-99.21983
Ejido Emiliano Zapata 2	90.51	24.042408	-99.2314
Ejido La Rosita 2	43.07	24.004097	-99.232506
Ejido Ceylán 1	34	23.97847	-99.14093
Ejido Acatlán	100	23.96543	-99.13133
Ejido Ceylán 2	34	23.9817	-99.14491

ARCO 3 consisted of 1 575.75 ha; 18 commercial orchards (Table 3) sown with Italian lemon trees. The average age of the trees was five years, the separation between the trees and rows was four and seven meters. The trees were watered with a micro-sprinkler irrigation system.

Table 3. Area and geographical location of the orchards of Italian lemon *Citrus limon* (L.) Burn f. of ARCO 3 located in the municipality of Güémez and Ciudad Victoria.

Commercial orchard	Area (ha)	Latitude	Longitude
Orchard La Viuda	55	23.915712	-99.09632
Orchard La Providencia 3	50	23.914999	-99.07397
Orchard Tres Sabinos	30	23.900478	-99.05132
Orchard Macabeos 4	56	23.925621	-99.02932
Ejido Esfuerzo del Campesino 2	2	23.937151	-99.12753
Orchard El Filosofo	53.25	23.916235	-99.06931
Orchard Najita	116	23.922585	-99.046234
Orchard La Ilusión	127.4	23.928032	-99.05204
Orchard La Querencia	60	23.917574	-99.050766
Ejido La San Juana 3	3.4	23.924044	-99.133865
Orchard El Contador	21	23.929514	-99.24125
Orchard El Cuatro	19	23.936605	-99.23088
Orchard El Jericob	74.61	23.885063	-99.13875
Orchard La Pomarosa	40	23.889816	-99.04195
Orchard Nuevo Guadalupe	181	23.884377	-99.031525
Orchard El 12	101.45	23.821293	-99.0759
Orchard Teresitas	251	23.899931	-99.183525
Orchard Laborcitas	334.64	23.851086	-99.09927

Record of the phenology of citrus trees

The record of the phenology of the Valencia orange and Italian lemon crops was carried out every eight days taking as a reference what was published by Robles and Delgadillo (2010); Lozano and Jasso (2012).

Monitoring of *D. citri* adults in the ARCOs

The adults were captured with yellow traps of 7.5 cm by 12.5 cm, stamped with a black grid of 1.5 cm and accompanied by a base covered with the adherent Stick Bug 50%®. In the orchards, the traps were placed based on the HLB campaign operating manual. Ten traps were placed on the periphery (South-East) and 10 traps in a transverse line to it, at a distance between traps of 12 m and 1.20 m from ground level. The traps were replaced every eight days and the number of adults was quantified in the laboratory. In ARCO 1, ARCO 2 and ARCO 3, 1 300, 980 and 360 traps were installed.

Statistical analysis

The number of *D. citri* individuals were grouped into six categories with ranges from 0 to 100 individuals, taking as a reference that the lowest number of adults captured per month is 20 and the largest is 134 (Flores *et al*., 2017). The categories of the population densities of the insect were: pest 1 (0 individuals), pest 2 (1 to 100 individuals), pest 3 (100 to 200 individuals), pest 4 (200 to 300 individuals), pest 5 (300 to 400 individuals) and pest 6 (400 to 500 individuals). The categories were associated with the phases of shooting, shooting-fruiting, shooting-flowering, shooting-development and dormancy of commercial orchards of Valencia orange (ARCO 1 and 2) and Italian lemon (ARCO 3).

Finally, the categories were subjected to a multivariate analysis of multiple correspondence (Benzécri, 1973; Greenacre, 1983). The analysis considers the percentage of inertia of the X and Y axes, interpreting the one with the highest percentage of inertia. The interpretation of the results consists of associating the categories with greater proximity considering frequency data without losing the identity or origin of the sampled sites (Rencher, 2002).

Results and discussion

Phenology of the Valencia orange and Italian lemon crops

The Valencia orange trees presented the following phenology: shoot production, flowering, fruit development, dormancy and fruiting. The production of shoots occurred from January to July and from October to December, fruiting occurred in January and December. Flowering began in late March and ended in the third week of April, the development phase began in late April and ended in July and the dormancy phase occurred in late July and ended in the first week of October. On the other hand, the Italian lemon trees presented the following phenology: shoot production, flowering, fruit development and fruiting. The production of shoots occurred all year round, fruiting occurred in January and December, flowering began at the end of March and ended in the third week of April and the development phase began at the end of April and ended in July.

Seasonal abundance of *D. citri* associated with the phenological phases of citrus fruits in the ARCOs

In 2015, the *D. citri* population presented two significant associations with the phenological phases in the three ARCOs X^2= 1664.78, d.f.= 121 (Figure 2). Populations of 1 to 100 individuals were associated with the shooting-development phase in ARCO 1 (A1). Whereas populations of 1 to 100 individuals were associated with the shooting-development, shooting-flowering and dormancy phases in ARCO 2 (A2) and population densities of 100 to 200 individuals were associated with the shooting phase in ARCO 3 (A3).

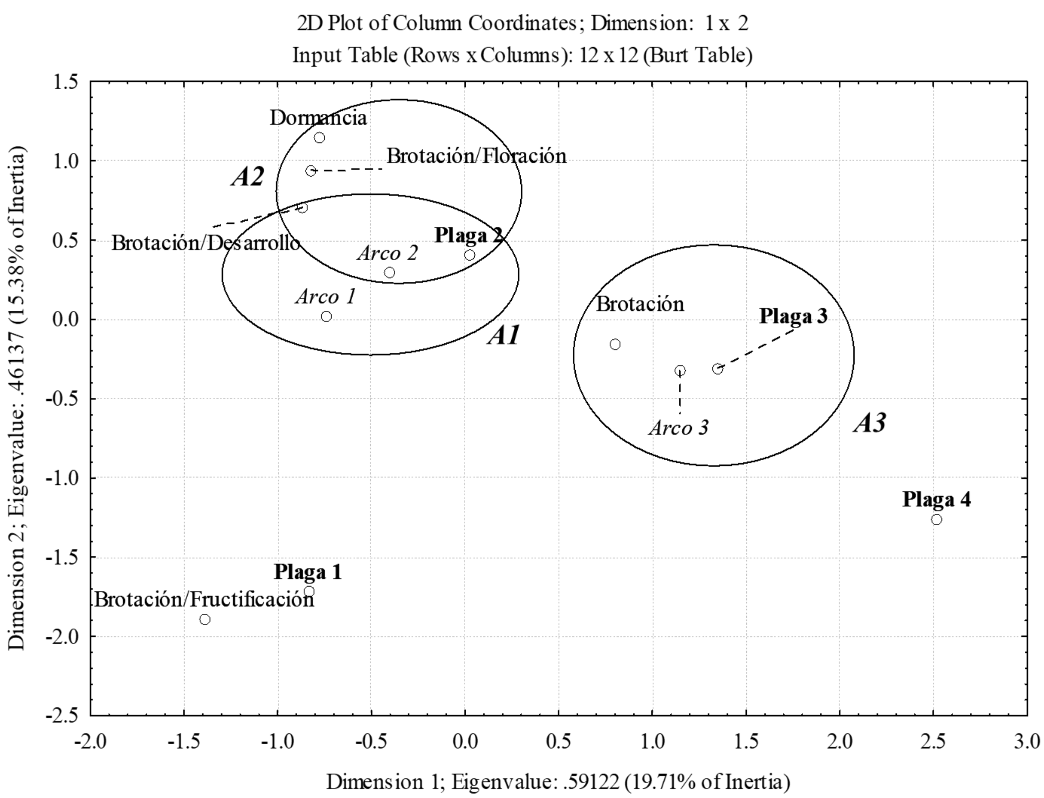

Figure 2. Analysis of the association of seasonal abundance of *Diaphorina citri* in the phenological phases of the Valencia orange and Italian lemon crops in three regional control areas in 2015. The categories of population densities of the insect were pest 1 (plaga 1) (0 individuals), pest 2 (plaga 2) (1 to 100 individuals), pest 3 (plaga 3) (100 to 200 individuals), pest 4 (plaga 4) (200 to 300 individuals), pest 5 (plaga 5) (300 to 400 individuals) and pest 6 (plaga 6) (400 to 500 individuals).

In 2016, *D. citri* adults showed a significant association with the phenological phases in the three ARCOs X^2= 1998.24, d.f.= 169 (Figure 3). Populations of 100 to 200 individuals were associated with the shooting-development phase in ARCO 1 (A1). Whereas populations of 400 to 500 individuals were associated with the shooting-development phase in ARCO 2 (A2). Similarly, densities of 400 to 500 individuals were associated with the shooting phase in ARCO 3 (A3).

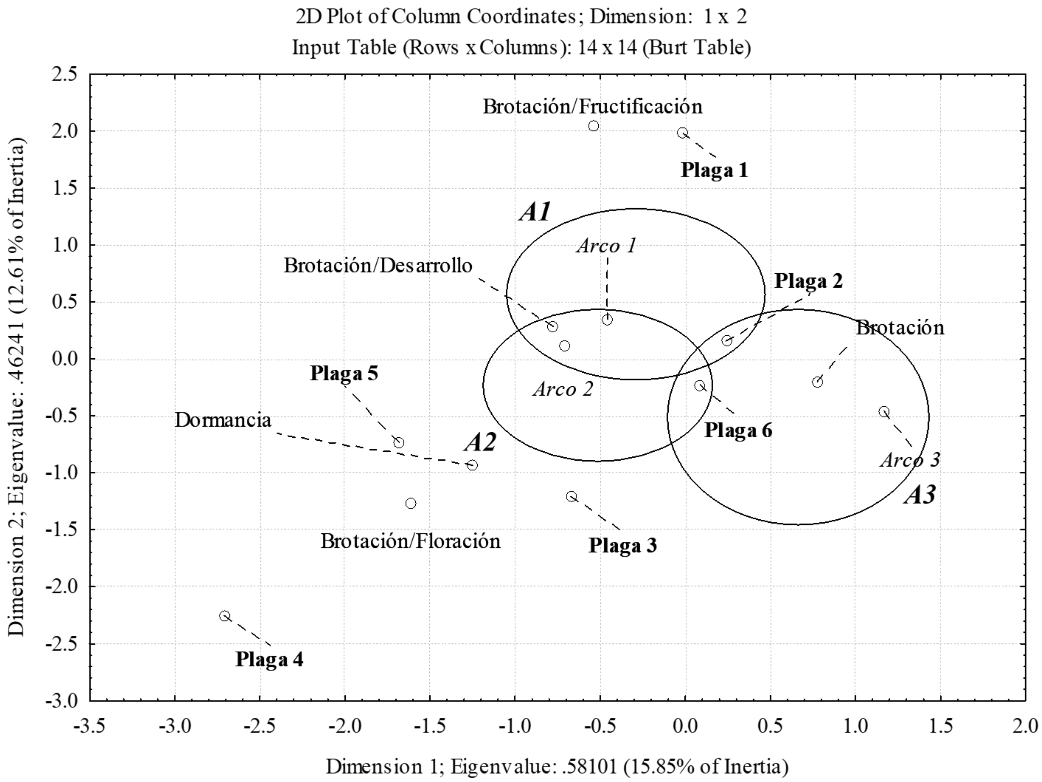

Figure 3. Analysis of the association of the seasonal abundance of *Diaphorina citri* in the phenological phases of the Valencia orange and Italian lemon crops in three regional control areas in 2016. The categories of population densities of the insect were pest 1 (plaga 1) (0 individuals), pest 2 (plaga 2) (1 to 100 individuals), pest 3 (plaga 3) (100 to 200 individuals), pest 4 (plaga 4) (200 to 300 individuals), pest 5 (plaga 5) (300 to 400 individuals) and pest 6 (plaga 6) (400 to 500 individuals).

In 2017, two associations occurred between the number of *D. citri* individuals with the phenological phases in the three ARCOs (X^2= 2087.39, df= 169) (Figure 4). The first association was carried out with population densities of 1 to 200 individuals in the shooting phase in ARCO 3 (A3). For the second association, populations of 200 to 300 individuals were associated with the shooting-flowering and shooting-development phases in ARCOs 1 and 2 (A1, A2).

In 2018, the population of *D. citri* presented significant associations with the phenological phases in the three ARCOs (X^2=2313.21, df= 196) (Figure 5). Population densities of 1 to 100 individuals were associated with the shooting, shooting-flowering, shooting-fruiting and dormancy phases in ARCO 1 (A1). Population densities of 100 to 200 individuals were associated with the shooting-development phase in ARCO 2 (A2). Finally, densities of 200 to 300 individuals were associated with the shooting phase in ARCO 3 (A3).

The abundance of *D. citri* was different in the ARCOs and in the years of study. According to Razeto (2005), the variety of the cultivar and the production of shoots in citrus trees influences the abundance of *D. citri*. In the present work, the difference in the abundance of *D. citri* was possibly due to the amount and period of production of shoots in the Valencia orange and Italian lemon crops, since the orange crop produces fewer shoots (38.70 shoots/month) per year than the lemon crop (673.5 shoots/month) (Medina *et al*., 2007; Chávez *et al*., 2016).

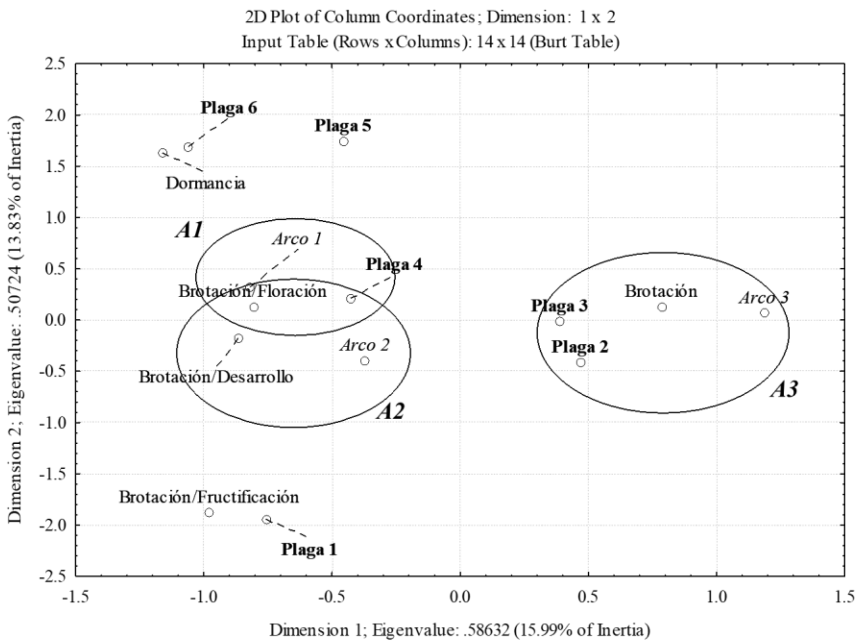

Figure 4. Analysis of the association of seasonal abundance of *Diaphorina citri* in the phenological phases of the Valencia orange and Italian lemon crops in three regional control areas in 2017. The categories of population densities of the insect were pest 1 (plaga 1) (0 individuals), pest 2 (plaga 2) (1 to 100 individuals), pest 3 (plaga 3) (100 to 200 individuals), pest 4 (plaga 4) (200 to 300 individuals), pest 5 (plaga 5) (300 to 400 individuals) and pest 6 (plaga 6) (400 to 500 individuals).

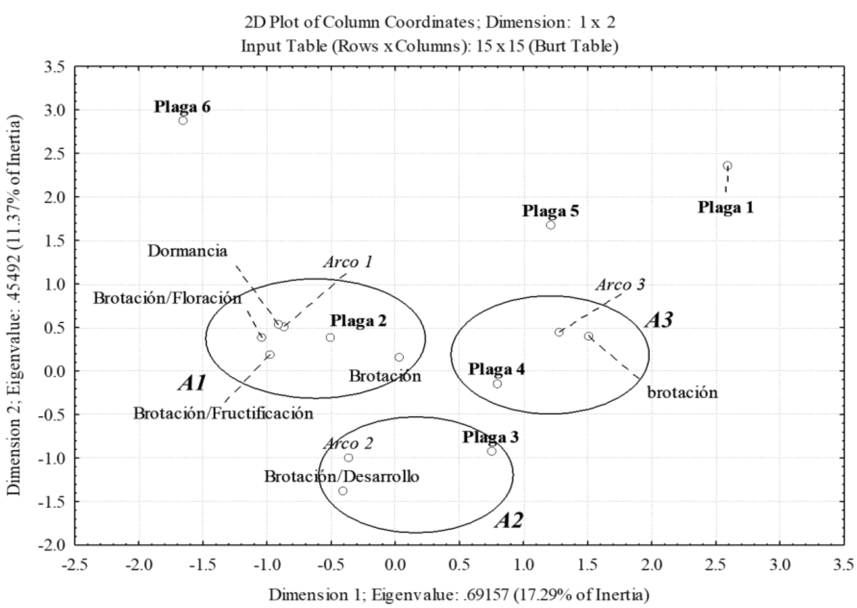

Figure 5. Analysis of the association of the seasonal abundance of *Diaphorina citri* in the phenological phases of the Valencia orange and Italian lemon crops in three regional control areas in 2018. The categories of population densities of the insect were pest 1 (plaga 1) (0 individuals), pest 2 (plaga 2) (1 to 100 individuals), pest 3 (plaga 3) (100 to 200 individuals), pest 4 (plaga 4) (200 to 300 individuals), pest 5 (plaga 5) (300 to 400 individuals) and pest 6 (plaga 6) (400 to 500 individuals).

In the ARCOs, the Valencia orange trees produced shoots from January to July and from October to December. Whereas the Italian lemon trees presented shoots all year round, possibly due to the micro-sprinkler irrigation system, use of fertilizers, pruning of the trees and because the lemon trees are younger (five years) than the orange trees (Pluke *et al*., 2008). While orange orchards do not have an irrigation system, fertilizer use is limited, and trees are on average 15 years old.

In the three years, the highest abundance of *D. citri* recorded in ARCO 3 was associated with the shooting phase of the Italian lemon crop. Whereas the greatest abundance recorded in ARCO 1 and ARCO 2 was associated with the shooting and shooting-development phases of the Valencia orange crop. In both crops, *D. citri* occurs in the phase of production and development of shoots, because adults and nymphs feed on them to mature their eggs and complete development (Ortega *et al*., 2013). Although adults also feed and survive for several months on fully developed leaves (Tsai *et al*., 2002; Fernández and Miranda, 2005; Stansly and Qureshi, 2007; Qureshi and Stansly, 2008). Hall *et al*. (2008) indicated that population peaks of *D. citri* occur when there are favorable environmental conditions and shoots availability.

In this regard, the Italian lemon crop produced shoots all year round (the greatest abundance of shoots was observed from December to March) and *D. citri* was present in the trees. Similarly, this has occurred in the Mexican lemon crop in the municipality of Apatzingán, Michoacán (Luna *et al*., 2018). *D. citri* is present throughout the year because the crop produces shoots constantly. However, the maximum population peak occurs in August because of the favorable environmental conditions for the insect. A similar result was determined in the Persian lemon (*C. latifolia*) in the municipality of Huimanguillo, Tabasco (García *et al*., 2013).

As in Mexican lemon orchards, this crop produces shoots constantly and the greatest abundance of *D. citri* occurs from March to July. Conversely, in the Mexican lemon in Acapulco, Guerrero, the smallest adult population of *D. citri* occurred in February, when the trees were in the shoot production stage. Whereas the greatest abundance occurred in April and November, in the stage of fruit set and fruit development, respectively. In this case, low rainfall (Botero *et al*., 2014) and optimal temperature played an important role in the occurrence of high population peaks of *D. citri* in trees (25 to 28 °C) (Liu and Tsai, 2000; Paris *et al*., 2017).

Contrary to the Italian lemon crop, the Valencia orange trees did not produce shoots all year round. However, the greatest abundance of *D. citri* occurred in the phase of production of shoots and in the development of these. A similar result was reported by Ortega *et al*. (2013) in Cazones, Veracruz. The authors recorded the periods of greatest abundance of *D. citri* in orange trees (*C. sinensis cv* Mars, *C. sinensis cv* Valencia) and sweet lime (*C. limetta*) from February to April and in July, which coincide with the phase of production and development of shoots.

Conclusions

During the years 2015 to 2018, the population size of *D. citri* was different in all three ARCOs. In this result, the phenology of the crop played an important role. Because the greatest abundance of the insect was recorded in the phase of production of vegetative shoots.

Acknowledgements

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