Article

Pumpkin curly leaf virus (SLCV): diagnosis, population dynamics of the vector and spatio-temporal distribution of the virus

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Abstract

The members of the genus begomovirus are transmitted by *Bemisia tabaci* and cause severe losses in cucurbitaceae. The objectives of the study were: i) to investigate the etiology of the disease caused by the pumpkin leaf curly virus; ii) know the population dynamics of the vector, compare the effectiveness of yellow sticky traps against yellow trays and determine the effect of the location of the traps, in monitoring *B. tabaci* during the crop development cycle; and iii) study the spatio-temporal distribution and severity of pumpkin virosis. The study was carried out in the experimental station of the Superior Agricultural College of the State of Guerrero, located in the Cocula Valley, Guerrero, Mexico. DNA was extracted from pumpkin leaves (*Cucurbita pepo*) var. Gray Zucchini with symptoms of virosis. The PCR was carried out in five samples with the generic oligonucleotides for begomoviruses, two PCR products were sequenced and a phylogenetic tree was generated. Pumpkin virus transmission test was performed, under controlled conditions. The population dynamics of *B. tabaci* was determined using sticky yellow traps and yellow trays. From the symptomatology, the severity percentage and the area under the disease progress curve (ABCPE) were obtained. PCR analysis, sequencing and phylogeny, confirmed the presence of the SLCV virus (KX620945.1-DAAV-1) in pumpkin. It was found that *B. tabaci* transmits to SLCV that significantly reduces plant growth. The sticky trap and sampling date influence the population fluctuation of *B. tabaci*. The incidence of *B. tabaci* and severity of SLCV were manifested in all the phenological stages of the crop and related to the temperature level.

Keywords: *Bemisia tabaci*, *Cucurbita pepo*, *Geminivirus*, severity.

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Introduction

The *Cucurbitaceae* family comprises many crops, including cucumbers, pumpkins, luffas, melons and watermelons (Pessarakli, 2016). Zucchini and squash (*Cucurbita* spp.) are the main horticultural species grown from fresh to tropical regions. The *C. pepo* species is one of the most widely cultivated, has a high economic impact and is mainly consumed as fresh vegetables because the fruit is of high nutritional value. Seeds are consumed in some regions and produce oil that is highly valued in central Europe (Paris *et al*., 2012).

Among the pests and diseases that significantly affect the yield and quality of the pumpkin fruit, the most important are those caused by phytopathogenic viruses, because they cause strong economic losses. More than 35 viruses have been identified in *Cucurbitaceae* (Ozaslan *et al*., 2006), including geminiviruses (family *Geminiviridae*) with circular single stranded DNA genome, encapsulated within isometric particles, which are differentiated by the vector, host range and genome organization (Varsani *et al*., 2017).

Begomoviruses, transmitted by whiteflies, have genomes with single or bipartite components (DNAs A and B) and infect dicotyledonous plants. DNA A encodes the envelope protein (AV1), as well as the proteins required for replication (AC1), gene regulation (AC2) and replication enhancement (AC3). DNA B is essential for the production of disease symptoms, but does not influence DNA replication (Rosen *et al*., 2015). The two gene products (BV1 and BC1), encoded by this component, are involved in the spread of the virus throughout the plant, the production of symptoms and the range of hosts (Ramesh *et al*., 2017).

In recent years, whitefly transmitted numerous begomoviruses have emerged as devastating pathogens, particularly in the tropics and subtropics, where they cause huge losses in various economically important crops (Al-Musa *et al*., 2008). Pumpkin leaf curly disease was first observed in pumpkin crops (*Cucurbita foetidissima*), in California during 1977 and 1978, as well as in Buffalo and Arizona (Rosemeyer *et al*., 1986). The incidence of SLCV was restricted to Central and North America; however, in 2003, the first record was made in Israel, where it caused severe epidemics with an incidence close to 100% (Antignus *et al*., 2003); subsequently, Idris *et al*. (2006) reported that SLCV-EG caused severe symptoms in pumpkin crops (*Cucurbita pepo*) in Egypt.

Likewise, in the Jordan Valley, symptoms similar to those caused by geminivirus were observed in *C. pepo* plants. (Al-Musa *et al*., 2008). Recently, SLCV-PAL was reported in Palestine, by Ali-Shtayeh *et al*. (2014), causing damage to pumpkin (*C. pepo*), watermelon (*Citrullus lanatus*) and cucumber (*Cucumis sativus*) plants. Monitoring and surveillance activities are key factors for the management of begomoviruses, because as the newly affected areas are identified, the targeting of control measures is facilitated; they also serve to determine disease propagation patterns and predict areas that could be affected in the future (Szyniszewska *et al*., 2017).

As in most insects, *Bemisia tabaci* colonizes leaves and the entire plant, at all stages of the crop. To determine the aggregation and behavior of *B. tabaci* populations within crop fields, it is important to determine the spatial and temporal structure of pest populations and define sampling

plans (Naranjo, 1996). Due to the importance of *B. tabaci* and the transmission of SLCV as limiting factors in commercial pumpkin production, it is necessary to understand broadly the population dynamics and severity of the SLCV virus.

This research had the following objectives: i) to investigate the etiology of the disease caused by the pumpkin leaf curly virus; ii) know the population dynamics of the vector, compare the effectiveness of yellow sticky traps against yellow trays and determine the effect of the location of the traps, in monitoring *B. tabaci* during the crop cycle; and iii) study the spatio-temporal distribution and severity of pumpkin virosis.

Materials and methods

Location

The study was carried out in the Experimental Field of the Superior Agricultural College of the State of Guerrero (CSAEGro), in Cocula, Guerrero, Mexico, located between the coordinates 18° 26' 27.20'' north latitude and -99° 65' 07.98'' longitude west, at 635 meters above sea level. Experimental cultivation of pumpkin var. Gray Zucchini was established between two tributaries of water, the San Juan River and Las Juntas irrigation water channel.

Virus detection of pumpkin curly leaf and phylogenetic analysis

From 15 symptomatic pumpkin leaves, DNA was extracted with the DNeasy Plant kit (QIAGEN®). Degenerated oligonucleotides prV324 (5-gccyatrtayagraagccmag-3') and CoPR (5-gangsatghgtrcadgccatata-3') were used to detect begomoviruses in general, and SLCV in particular, these oligonucleotides bind to begomovirus DNA and amplify fragments of approximately 570 bp (Wyatt and Brown, 1996; Yongping *et al*., 2008). The PCR reactions were performed following a program with an initial temperature of 94 °C for 4 min, followed by 35 cycles of 94 °C 60 s, 50 °C 45 s and 72 °C 45 s; with a final extension temperature of 72 °C for 6 min.

A Thermo™ thermocycler (Thermo Scientific, Wilmington, DE) was used for DNA amplification and the visualization of the amplified products was carried out by electrophoresis in 1% agarose gels at 62 volts for 5 min, followed by 100 volts by 40 min, and were observed in a UVMR light transilluminator (Labnet, Edison, NJ). Two representative samples of the PCR products were sequenced, and the sequences obtained were compared with the database of the Gene Bank (GenBank) of the National Center for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov/).

The consensus sequences were edited and assembled with the CAP (Contig Assembly Program) option of the BioEdit 7.2.5 Software (Tom Hall Ibis Biosciences) (Hall, 2004). In the evolutionary analysis, all consensus sequences were aligned with the ClustalW program (Thompson *et al*., 1994) included in the MEGA 7 software (Kumar *et al*., 2016). The phylogenetic reconstructions of the data were performed using the maximum parsimony method, using the Subtree-Pruning-Regrafting algorithm, search option (level=1) with the initial tree by random addition (10 repetitions) and the

missing spaces were considered as complete deletions. To calculate the confidence values of the tree clades, a bootstrap test was performed with 1 000 repetitions (Felsenstein, 1985). The sequence obtained was deposited in the NCBI GenBank database.

SLCV virus pathogenicity

The SLCV transmission test, by whitefly (*B. tabaci*), was performed on pumpkin (*C. pepo*) var. Gray Zucchini during the period February-March 2015. This was carried out in cages made with two expanded polystyrene glasses of 1 L capacity, with windows covered with organza fabric.

On March 15, adults of *B. tabaci* were collected using an oral sucker, in a pumpkin crop with virosis syndrome established in the experimental field of the Superior Agricultural College of the State of Guerrero. 30 insects were placed per cage, which contained a pumpkin plant. For this, five repetitions and two witnesses were included. They were inspected for 21 days to detect the appearance of symptoms of virosis. From the infected leaves, obtained from the cages, one was selected and DNA was extracted and by PCR the virus was detected using the degenerate oligonucleotides prV324/CoPR.

Population fluctuation of *B. tabaci* and spatio-temporal analysis of the severity of SLCV

In February 2015, pumpkin var. Gray Zucchini in a batch of 2 880 m^2, where a census was carried out in 20 quadrants with 10 rows and 20 plants per row. Traps were distributed in the four cardinal points and in the center of the lot by an experimental design of randomized complete blocks with two repetitions.

The types of traps were: a) 18 × 24 cm yellow sticky trap placed on a metal support 1 m high and 1 cm in diameter; and b) yellow tray trap of 17 and 12 cm of upper and lower diameters and 10 cm deep with the capacity to store 1 L of water. The traps were inspected 12 times, every three and four days, except in the last evaluation where the interval was 10 days. The number of adults of whiteflies in them was counted.

The data of these variables were analyzed with the SAS 9.4 statistical program. The severity of virosis was calculated using the Van Der Plank (1963) scale and five evaluations were performed every eight days. To determine the effect generated by this phytopathological problem during the entire pumpkin growing cycle and compare the epidemic of virosis, the area under the disease progress curve (ABCPE) was calculated using the severity percentage data (Campbell and Madden, 1990). The data analysis included maps of the spatial distribution, such as response surfaces and contour curves (isolines) obtained using the kriging interpolation method, using the SAS 9.4 statistical program.

Results and discussion

SLCV detection and phylogenetic analysis

Severe symptoms of pumpkin leaf curl were observed at the experimental site. PCR and sequencing analyze revealed that the leaves with virosis symptoms obtained in the Cocula Valley, Guerrero Mexico, were infected with SLCV. The virus genome sequence obtained in this work was deposited in the NCBI GenBank (accession KX620945.1 DAAV-1).

El-Dougdoug *et al*. (2009) identified the SLCV in *C. pepo* var. Eskandrani, using the same oligonucleotides as in the present investigation; reported that amplified DNAs were 480 bp in size. The phylogenetic analysis showed that the SLCV found in Guerrero, Mexico is closely related to the SLCV accessions found in Cairo (DQ285019) and The Imperial Valley of California (USA) (DQ285016), with bootstrap reliability value of 96%. In addition, the accessions of American and Middle Eastern origin differed from the group of Asian accessions (AM260206, EU47910 and EFQ99774) (74%) (Figure 1).

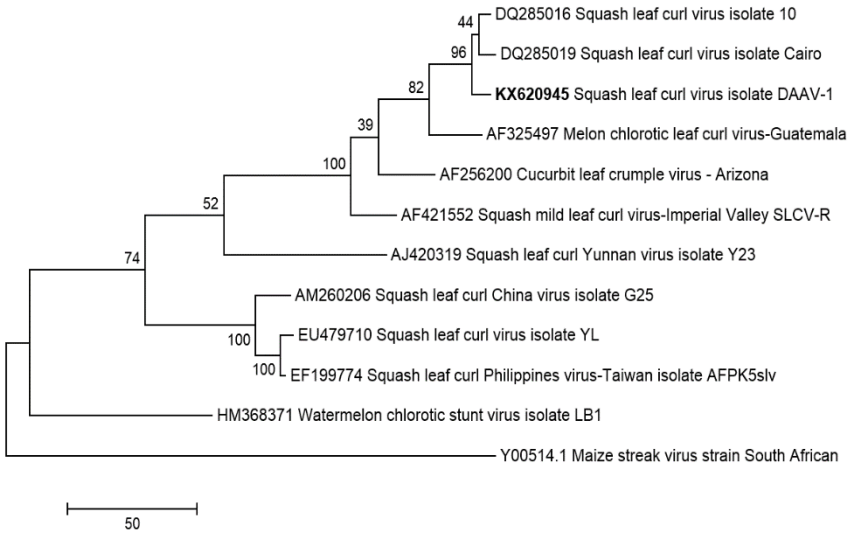

Figure 1. Phylogenetic tree obtained with the maximum parsimony method using the Subtree-Pruning-Regrafting algorithm. The reliability values of the nodes were formed with 1 000 bootstrap resamples. Bold access corresponds to the variant evaluated in this study.

SLCV pathogenicity

The results of this test indicated that whitefly transmitted by pumpkin SLCV is pathogenic. Symptoms manifested at 8 days after the introduction of whitefly in the cages. SLCV infection causes leaf curl, growth retardation, epinastia, intervenal chlorosis and mottled leaves (Figure 2). These results coincide with what was reported by Idris *et al*. (2006), who studied the SLCV virus in cucurbits, in Jordan and Egypt and found that the infection affected leaf size and reduced plant growth by 40%.

Also, Sobh *et al*. (2012) reported that SLCV infection caused severe symptoms of pumpkin leaf curl (*Cucurbita* sp.) Camelia F1 variety, at 2 weeks after inoculation; that is, they were presented in double the time compared to the results of the present trial. Similarly, they agree with Taha *et al*. (2016), which confirmed that the SLCV virus is transmitted by whitefly and induces severe growth retardation, leaf curly and causes serious losses in the production of cucurbitaceae. In addition, it was determined that the amplified product of the SLCV, obtained from infected pumpkin leaves of the experiment, had a size of 464 bp. The sequence was deposited in the GenBank of the NCBI (accession KX620948.1 DAAV-2).

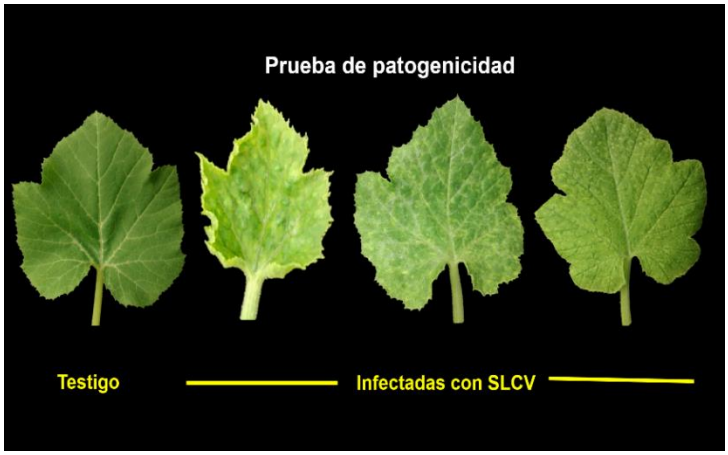

Figure 2. SLCV pathogenicity test in pumpkin var. Gray Zucchini plants. Healthy leaf (left) and leaves infected with the pumpkin curly leaf virus with different severity (right).

Population fluctuation of *B. tabaci*

The number of whitefly adults showed significant differences in the majority of the samples, due to the type of trap. It was found that, at all sampling dates, the sticky trap was more effective than the tray with water, to capture these insects, whose averages ranged from 7.4 (sample 2) to 379 (sample 11).

The analysis of the cumulative sampling of all samples indicated that 2 082 adults (data not shown) were captured in the sticky trap, compared to the tray type trap (416 specimens). The location of the trap only significantly influenced the amount of insects captured in samples 1, 3 and 6. In the first samples (1 and 3), the averages indicate that the largest populations of flies were recorded at the West site; however, in the final evaluations, the highest insect density occurred in the traps of the East and North.

The highest accumulated amount of *B. tabaci* was obtained on the East side, with 538 individuals, the lowest value was 443 and was found on the South site (Table 1). On the other hand, there were significant differences between the sampling dates (Figure 3), since the populations varied in a range of 26 (date 2) to 437 insects (date 11). Pest management programs should be based on accurate knowledge of the main factors responsible for changes in the dynamics of the target insect population.

Table 1. Comparison of means of the number of whiteflies in the type of trap, the date of sampling and the cardinal location.

Sampling/date	Number of whitefly adults		DSH	Probability
	Yellow tray with water	Yellow sticky trap		
1 (11/02/15)	0 b†	7.4 a	1.44	<0.0001
2 (14/02/15)	6 a	22 a	2.88	0.2453
3 (18/02/15)	42 a	66 a	25	0.2825
4 (21/02/15)	44 b	96 a	31	0.0003

Sampling/date	Number of whitefly adults		DSH	Probability
	Yellow tray with water	Yellow sticky trap		
5 (25/02/15)	43 b	146 a	5.97	0.0032
6 (28/02/15)	52 b	156 a	4.11	0.0002
7 (04/03/15)	60 b	214 b	4.03	<0.0001
8 (07/03/15)	15 b	298 a	6.28	<0.0001
9 (11/03/15)	62 b	224 a	9.56	0.0036
10 (14/03/15)	16 b	236 a	8.95	0.0003
11 (18/03/15)	58 b	379 a	7.45	<0.0001
12 (28/02/15)	12 b	168 a	9.99	0.0060
Location		Averages of the 12 samples		
North		497 a††		
South		443 a		
East		538 a		
West		506 a		
Center		514 a		

†, (††)= values with equal letters in the same row (column) are not statistically different (Tukey, α= 0.05).

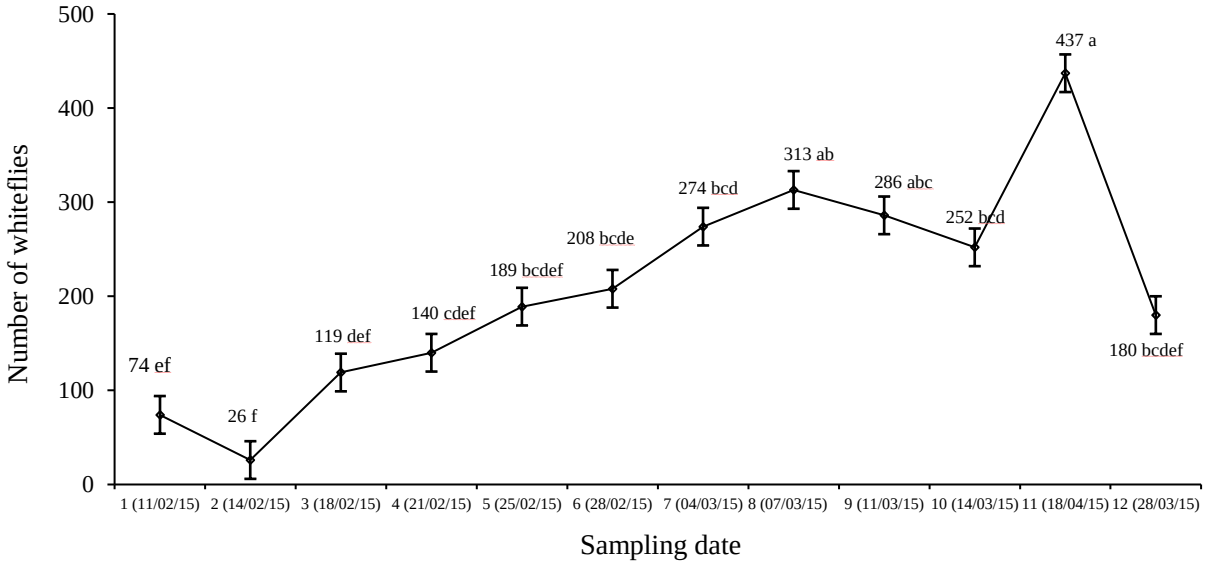

Figure 3. Number of whiteflies according to the sampling date.

The biological cycle parameters estimated in different biotic and abiotic conditions provide the basic tools to understand the changes and behavior of pest species. The species *B. tabaci* is a poikilothermic organism, that is, the temperature influences the biological cycle; therefore, it is important to consider this factor to explain the ecology of the population. In this regard, Drost *et al*. (1998) reported that, in addition to temperature, host plants and biotypes influence the biology of *B. tabaci*.

The results obtained confirm what Curnutte *et al*. (2014), who mention that oviposition, the survival of nymphs and the reproduction of *B. tabaci*; were significantly affected by the temperature, since the total reproductive capacity decreased 36.4% at 33 °C, also, they noted that 28 °C was the favorable temperature for whitefly development; however, they determined that the optimum temperature for the reproduction of *B. tabaci* fluctuates from 28 to 33 °C.

In this study, it was found that during the sampling the average temperature increased, which in combination with the phenology of the crop favored the increase in the reproductive rate of *B. tabaci*. It has been shown that temperatures from 30 to 32 °C favored the development of *B. tabaci* (Bonato *et al*., 2007; Curnutte *et al*., 2014). In this regard, Quintela *et al*. (2016) found that the maximum population of the *B. tabaci* biotype B in corn (*Zea mays*) coincided with high temperatures in Brazil.

These findings are similar to those obtained in the present study, where the population of the pest increased in relation to the increase in temperature. On the contrary, Bonato *et al*. (2007) reported that the temperature of 32.5 °C was the optimum for the development of nymphs of *B. tabaci* (biotype Q); in addition, they determined that total fertility (eggs per female) varied from 105.3 (21 °C) to 41 (35 °C) and that longevity decreased as the temperature increased. Similarly, they argued that the association between temperature and biological cycle parameters is useful for predicting the effect of temperature on population dynamics.

Spatio-temporal analysis of the severity of SLCV

The average severity percentage (PS) recorded; through, the evaluation dates fluctuated between 5.14 and 73.28. It was determined that, from evaluation 5, the average PS remained stable, while the temperature registered an increasing trend, which was related to the severity of the SLCV (Figure 4).

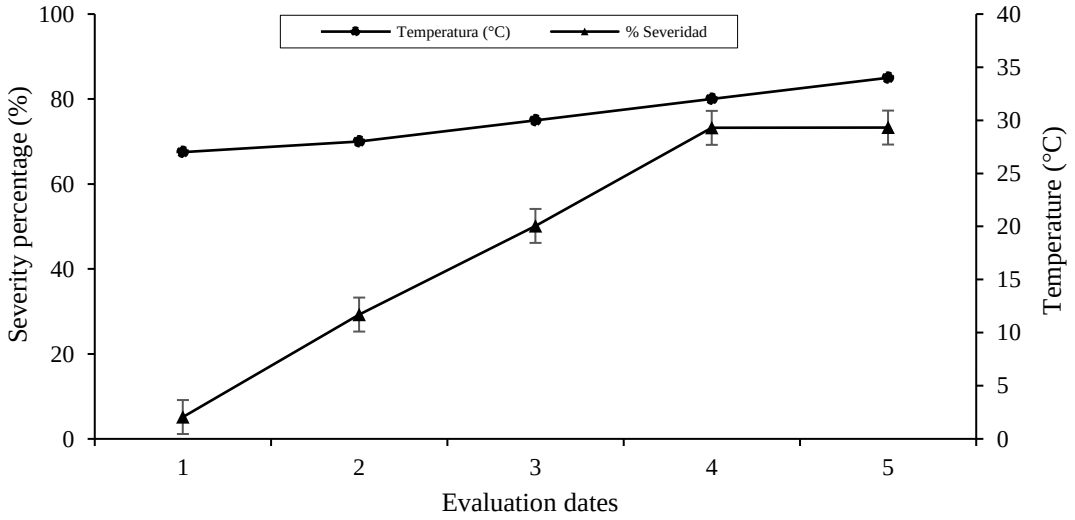

Figure 4. Relationship of average temperature and severity of SLCV in pumpkin var. Gray Zucchini plants, in five evaluations in Cocula, Guerrero.

The relationship between the severity and the temperature observed in this work coincides with that reported by Ali *et al*. (2014) who reported that temperatures between 37.16 and 37.78 °C led to the development of the disease; however, the minimum average temperature of 27.43 °C was favorable for the incidence of geminivirus in cotton and showed significant effect during two consecutive cycles of culture. The combined analysis, through the evaluation dates, indicated that quadrants 7 to 10 and 16 to 20 had a higher PS (47.88 to 54.87%).

It was found that ABCPE recorded significant differences ($p< 0.0001$) between quadrants and grooves; the highest incidence of disease occurred between quadrants 7 to 10 and 16 to 20, with values of 1431.72 to 1674.41; likewise, it was observed that the severity in quadrants 1 to 6 and 11 to 15 was less than 50%, while quadrants 17 and 19 had the highest severity of virosis in the pumpkin crop. The PS and ABCPE were lower in quadrants 2 to 5, with values of 39.9 to 40.23% and 1049.78 to 1134.66, respectively, indicating that the SLCV-induced epidemics were high intensity, monitored through changes in the severity of virosis (active and progressive infection) (Figure 5).

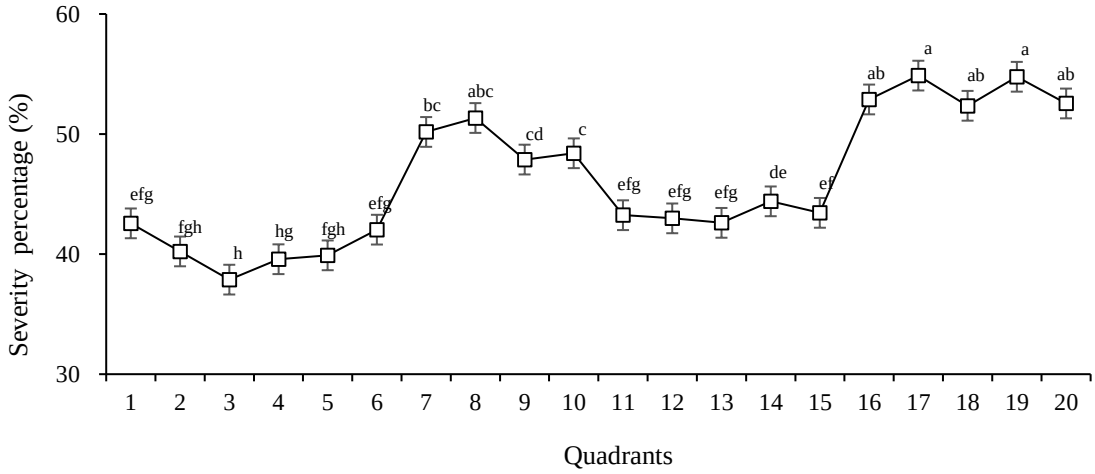

Figure 5. Percentage of severity of SLCV per quadrant in pumpkin var. Gray Zucchini plants, in different evaluations in Cocula, Guerrero.

A pronounced gradient of the PS of SLCV was observed in the quadrants north and northeast of the study area, compared to the central and southeast parts that were little affected (Figure 6). In the contour plot, light areas indicate a lower intensity of severity, while dark areas indicate greater severity of virosis. An apparent shore effect attributable to the source of primary inoculum was perceived, perhaps by perennial weeds from the San Juan River bank; but no uniform distribution of the disease was detected in the culture.

The distribution pattern of the SLCV in the study area revealed a strong spatial association between the severity of the virosis and the incidence of *B. tabaci*, since the first one increased in the western part during the crop cycle. Similar results reported a close spatial association between the incidence and severity of the virus in several cycles, which tended to increase towards a specific and localized part of the study area, during the spatial analysis and temporal change in the pandemic of a cassava geminivirus in northwestern Tanzania (Szyniszewska *et al*., 2017).

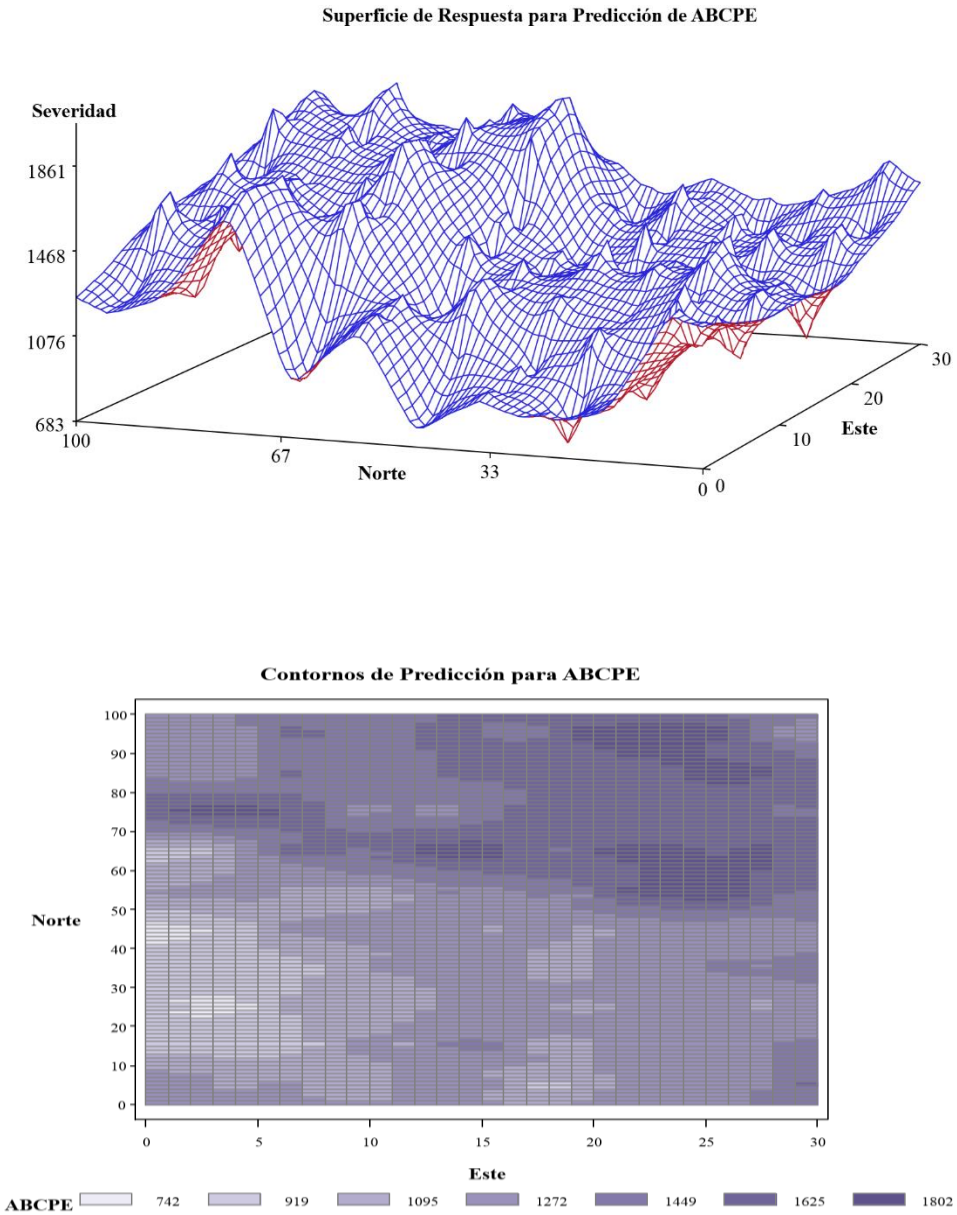

Figure 6. Response surface map and plane of contour curves or isolines, of the dispersion and frequency of the severity distribution of the SLCV in pumpkin var. Gray Zucchini

In the present study, the spatial distribution of the incidence and severity of the SLCV provided information to explain the arrival and distribution of *B. tabaci* populations as a virus vector, from weeds present in areas adjacent to the San Juan River, where the humidity it is permanent and favors the permanent incidence of weeds that function as reservoirs of *B. tabaci*.

These observations have been supported by the report by Mubin *et al*. (2009), who argue that weeds act as begomovirus reservoirs and are responsible for the appearance of virosis at the beginning of the crop cycle, becoming severe epidemics of cultivated plants; while, during the

dry season, weeds play the crucial role in the spread of various viruses, they act in the redistribution of the primary inoculum and serve as a refuge for vector insects, which are an important part in the epidemiology of begomoviruses (Zaidi and Mansoor, 2017).

In another study, it was mentioned that the most significant variation pattern in the incidence of whitefly was recorded among quadrants during the same season, in addition to the severity of a begomovirus, it is associated with environmental conditions, since during one of the cycles of experimentation, a fresh climate was presented and the severity of virosis was lower, compared to the cycle that had a warm and dry climate and therefore, the severity increased over the spatial and temporal distribution of cassava begomovirus (Szyniszewska *et al*., 2017). The findings obtained in this research corroborate the results published by Legg (2010), who confirmed that the changes in incidence of whitefly and the patterns of dissemination of virosis, are closely correlated.

Conclusions

The PCR analysis, sequencing and phylogeny, confirmed the incidence of Pumpkin Curly Leaf Virus (SLCV) in this same species. *Bemisia tabaci* transmits to SLCV, which significantly reduces plant growth. The yellow sticky trap was more efficient than the yellow tray. Sampling dates were useful to detect the temporal population fluctuation of *B. tabaci*. The incidence of *B. tabaci* and the severity of SLCV were presented in all the phenological stages of the crop with spatial variation in the experimental lot and were related to the temperature level.

Cited literature

Ali, S.; Khan, M.; Talib, S. S.; Atiq, M. and Hannan, A. 2014. Algodon leaf curl virus predictive model based on environmental variables. *In*: Ahmad, P.; Wani, M.; Azooz, M. and Phan, Tran L. S. (Eds.). Crop improvement in the age of Climate Change. Springer, New York.

Ali-Shtayeh, M. S.; Jamous, R. M.; Hussein, E. Y.; Mallah, O. B. and Abu-Zeitoun, S. Y. 2014. Squash leaf curl virus (SLCV): a serious disease threatening cucurbits production in Palestine. Virus Genes. 48:320-328.

Al-Musa, A.; Anfoka, G.; Misbeh, S.; Abhary, M. and Ahmad, F. H. 2008. Detection and molecular characterization of Squash leaf curl virus (SLCV) in Jordan. J. Phytopathol. 156:311-316.

Antignus, Y.; Lachman, O.; Pearlsman, M.; Omer, S.; Yunis, H.; Messika, Y.; Uko, O. and Koren, A. 2003. Squash leaf curl geminivirus-a new illegal immigrant from the western hemisphere and threat to cucurbit crops in Israel. Phytoparasitica. 31:415-415.

Bonato, O.; Lurette, A.; Vidal, C. and Fargues, J. 2007. Modeling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). Physiol. Entomol. 32:50-55.

Campbell, C. L. and Madden, L. V. 1990. Introduction to plant disease epidemiology. John Wiley & Sons. New York. 532 p.

Curnutte, L.; Simmons, A. and Abd-Rabou, S. 2014. Climate change and *Bemisia tabaci* (Hemiptera: Aleyrodidae): impacts of temperature and carbon dioxide on life history. Ann. Entomol. Soc. Am. 107:933-943.

Drost, Y. C.; Van Lenteren, J. C. and Van Roermund, H. J. W. 1998. Life-history parameters of different biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to temperature and host plant: a selective review. Bulletin Entomol. Res. 88:219-229.

El-Dougdoug, K. A.; Hayam, S. A. E.; Ibtisam, A. H.; Entsar, A. A.; Asmaa, F. and Abd, E. M. 2009. Identification of squash leaf curl virus (Egyptian isolate). Australian J. Basic Appl. Sci. 3(4):3470-3478.

Felsenstein, J. 1985. Confidence limits of phylogenies: an approach using the bootstrap. Evolution. 39:783-791.

Hall, T. 2004. BioEdit version 7.0.0. Isis pharmaceuticals Inc. 192 p. http://www.mbio.ncsu.edu/BioEdit/biodoc.pdf.

Idris, A. M.; Abedl-Salam, A. and Brown, J. K. 2006. Introduction of the new world squash leaf curl virus to squash (*Cucurbita pepo*) in Egypt: a potential threat to important food crops. Plant Dis. 90:1262-1667.

Kumar, S.; Stecher, G. and Tamura, K. 2016. Mega7: molecular evolutionary genetics analysis version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33(7):1870-1874.

Legg, J. P. 2010. Epidemiology of a whitefly-transmitted cassava mosaic geminivirus pandemic in Africa. *In*: bemisia: bionomics and management of a global Pest. Stansly, A. and Naranjo, S. E. (Eds.) Springer, Dordrecht, Heidelberg, London, New York. 233-257 pp.

Mubin, M.; Briddon, R. W. and Mansoor, S. 2009. Diverse and recombinant DNA betasatellites are associated with a begomovirus disease complex of *Digera arvensis*, a weed host. Virus Res. 142:208-212.

Naranjo, S. E. 1996. Sampling Bemisia for research and pest management applications. *In*: Gerling, D. and Mayer, R. T. (Eds.). Bemisia: taxonomy, biology, damage, control and management. Intercept, Andover, UK. 209-224 pp.

Ozaslan, M. T.; Aytekin, B.; Bas, I.; Hali-Kilic, I.; Didem, A. and Dag, D. S. 2006. Virus diseases of cucurbits in Gaziantep-Turkey. Plant Pathol. J. 5(1):24-27.

Paris, H. S.; Lebeda, A.; Kristkova, E.; Andres, T. C. and Nee, M. H. 2012. Parallel evolution under domestication and phenotypic differentiation of the cultivated sub species of *Cucurbita pepo*. Eco. Bot. 66(1):71-90.

Pessarakli, M. 2016. Handbook of cucurbits, growth, cultural practices, and physiology. CRC Press, Taylor & Francis Publishing Group.

Quintela, E. D.; Abreu, A. G.; Lima, D. J. F.; Mascarin, G. M.; Dos Santos, J. B. and Brown, J. K. 2016. Reproduction of the whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) B biotype in maize fields (*Zea mays* L.) in Brazil. Pest Management Sci. 72(11):2181-2187.

Ramesh, S. V.; Sahu, P. P.; Prasad, M.; Praveen, S. and Pappu, H.R. 2017. Geminiviruses and plant hosts: a closer examination of the molecular arms race. Viruses. 9(9):256-261.

Rosemeyer, M. E.; Brown, J. K.and Nelson, M. R. 1986. Five viruses isolated from field-grown buffalo gourd, *Cucurbita foetidissima* HBK, a potential crop for semi-arid lands. Plant Dis. 70:405-409.

Rosen, R.; Kanakala, S.; Kliot, A.; Cathrin-Pakkianathan, B.; Farich, BA.; Santana-Magal, N.; Elimelech, M.; Kontsedalov, S.; Lebedev, G.; Cilia, M. and Ghanim, M.2015. Persistent, circulative transmission of begomoviruses by whitefly vectors. Curr. Opin. Virol. 15:1-8.

Sobh, H. J.; Samsatly, M.; Jawhari, C.; Najjar, A. H. and Abou-Jawdah, Y. 2012. First report of squash leaf curl virus in cucurbits in Lebanon. Plant Dis. 96(8):1231-1236.

Szyniszewska, A. M.; Busungu, C.; Boni, S. B.; Shirima, R.; Bouwmeester, H. and Legg, J. P. 2017. Spatial analysis of temporal changes in the pandemic of severe cassava mosaic disease in Northwestern Tanzania. Phytopathology. 107:1229-1242.

Taha, O.; Farouk, I.; Abdallah, A. and Abdallah, N. A. 2016. Use of post transcription gene silencing in squash to induce resistance against the Egyptian isolate of the squash leaf curl virus. Int. J. Genomics. Article ID 6053147, 9 pages http://dx.doi.org/10.1155/2016/6053147.

Thompson, J. D.; Higgins, D. G. and Gibson, T. J. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Van Der Plank, J. E. 1963. Plant disease: epidemic and control. Academic Press, New York, London.

Varsani, A.; Roumagnac, P.; Fuchs, M.; Navas, C.J.; Moriones, E.; Idris, A.; Briddon, R.W.; Rivera, B. R.; Zerbini, F. M. and Martin, D. P. 2017. Capulavirus and Grablovirus: two new genera in the family Geminiviridae. Arch. Virol. 162:1819-1831.

Wyatt, S. D. and Brown, J. K. 1996. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology. 86:1288-1293.

Yongping, Z.; Weimin, Z.; Huimei, C.; Yang, Q.; Kun, S.; Yanhui, W.; Longying, Z.; Li, Y. and Zhang, H. 2008. Molecular identification and the complete nucleotide sequence of TYLCV isolate from Shanghai of China. Virus Genes. 36:547-551.

Zaidi, S. S. and Mansoor, S. 2017. Viral vectors for plant genome engineering. Front. Plant Sci. 8:539-544. doi: 10.3389/fpls.2017.00539.