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Potential causal factors of "heart-shaped cladode" malformations in cactus pear (*Opuntia ficus-indica* (L.) Miller)

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Abstract. The prevalence and severity of pests and diseases are factors that limit productivity and quality in commercial cactus pear plantations in Mexico. Recently, in several cactus pear producing regions the appearance of diverse symptoms associated with "heart-shaped cladodes" has been registered. This disease is characterized by the loss of apical dominance of vegetative shoots leading to abnormal cladode growth. Besides the aesthetic damage, vegetative and floral buds disappear in the zone of invagination, thus reducing productivity. Because to date the causal agent is unknown, this study was conducted to analyze three possible agents, damage by insects, physical damage and phytoplasmas that could possibly cause the symptomatology of "heart-shaped cladode", aiming to constitute a base line for future studies. The study was conducted under semi-controlled conditions and in the field, using *Opuntia ficus-indica* cv. Villanueva plants and cladodes. To rule out causal agents that occur naturally in cactus pear productive systems, the following characteristics were analyzed: detected presence of *Diabrotica undecimpunctata*, simulation of physical damage by puncturing 4- and 13-day-old shoots with a needle, and detection of phytoplasmas with PCR and RFLP. The results of the study showed that, although it feeds on shoots, *D. undecimpunctata* does not cause the symptoms. The puncture with a needle on the apical part promoted the presence of symptoms. The PCR and RFLP analyses detected the presence of phytoplasmas on both symptomatic and asymptomatic shoots. For this reason, it was not possible to conclude that phytoplasmas are the causal agents of heart-shaped cladode.

Key words: Nopal, Cactus pear, diseases, physiopathologies, etiology

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Introduction

The cactus pear (*Opuntia* spp.) is an important plant resource for Mexico as well as for other countries. The fruits and tender shoots (Nefzaoui, 2018), known as nopalitos, of some species are used as human food. The cladodes are also used as forage (Batista-Dubeux *et al*., 2021), and for rearing cochineal (*Dactylopius coccus*) to produce carminic acid (Zacarías-Alvarado *et al*., 2020).

For commercial production, it is necessary to provide adequate agronomic management to the plantations, being the phytosanitary aspect one of the factors that affect yield and quality of both fruits and cladodes (Méndez-Gallegos *et al.,* 2013; Ochoa *et al*., 2015a).

The entomofauna associated with the genus *Opuntia* is varied and numerous. Up to 122 insects that can damage all the plant structures have been found feeding on roots, stems, cladodes, flowers, and fruits (Moran, 1980). Mena-Covarrubias (2018) reported the presence of 12 insects that are pests of primary importance in cactus pear. Outstanding among these, for the severity of the damage, are *Dactylopius opuntiae* Cockerell (Mazzeo *et al*., 2019) and *Cactoblastis cactorum* Berg (Pemberton & Cordo, 2001). In addition, cactus pear is attacked by various types of pathogens, such as fungi, bacteria, viruses and phytoplasmas, which is favored by the high moisture content of the cladodes and the environmental conditions of each region (Zimmermann & Granata, 2002; Swart & Swart, 2003). In this regard, Granata *et al*. (2018) reported the prevalence of at least 15 diseases of global importance, including a symptomatic complex caused by phytoplasmas and viruses. In Mexico, cladode thickening, or “macho plant”, caused by the phytoplasma 16SrXIII-Mexican (Suaste-Dzul *et al*., 2012) and black spot *Pseudocercospora opuntiae* (Hernández-Sánchez *et al*., 2014; Ochoa *et al*., 2015b) are considered the major diseases in the main producer-regions.

One of the most recent phytosanitary problems is related to apical and lateral malformations on the cactus pear cladodes that appear in the initial growth stages of the vegetative shoots, in both wild populations and commercial cactus pear plantations in Mexico and elsewhere. The visible symptoms have been grouped under the term “heart-shaped cladode” because of the typical shape they acquire, with some variations (Mendoza-Orozco *et al*., 2018). This malformation causes a reduction in cladode size, photosynthetic area, and number of floral and vegetative buds, decreasing production of fruits, tender shoots, forage, and seed plants for new plantations. Hernández-Pérez *et al.* (2009) published, to our knowledge, the first report of the symptoms of heart-shaped cladode, associating them with phytoplasmas belonging to the subgroup 16srll. However, later investigations did not associate the malformation with these pathogens. The malformations in plants have been commonly attributed to infections by viruses, viroids and phytoplasmas (Spallino *et al*., 2017; Singh *et al*., 2018). However, heart-shaped cladode malformations are not entirely in accord with symptoms associated with phytoplasmas in other studies (Granata *et al*., 2006; Hernández-Pérez *et al*., 2009; Fucikovsky *et al*., 2011; Omar & Foissac, 2012; Suaste-Dzul *et al*., 2012; Martínez-Salgado *et al*., 2020). A group of pathogens that have recently received attention for their possible sanitary impact are geminiviruses that consist of circular single-chain DNA molecules. Among these geminiviruses is the Opuntia virus1 (OpV1) that infects different species of cactus, including *Opuntia* spp. (Fontenele *et al*., 2020).

The preliminary studies on the etiology of heart-shaped cladodes have been conducted to determine the causal agent(s), biotic or abiotic, of those malformations. Nevertheless, there is no conclusive evidence that such malformations are due to some physical damage at initial growth stages of the cladodes, or to a combination of factors (Mendoza-Orozco *et al*. 2018). For this reason, the objective of this study was to identify possible causes of these malformations using procedures designed to consider the symptoms observed in affected cladodes, as well as previous studies on the etiology of diseases in *Opuntia* spp.

Materials and Methods

Experimental procedure and plant material

The study was conducted under semi-controlled greenhouse conditions, and in the field. The physical damage to cladodes was promoted to rule out potential causal agents. The *Opuntia ficus-indica* cv. Villanueva adult cladodes free of pests, diseases, and damage was used. The selected cladodes were detached from the mother plant using tools previously disinfected with 6% sodium hypochlorite to prevent possible dispersion of pathogenic agents and ensure independence of the results. The cut cladodes were left to scar for 15 days; after this time, they were planted in 20-L pots containing peat moss. Substrate moisture was maintained with weekly watering.

Damage by Diabrotica undecimpunctata

The individual cladodes with 5, 9, and 13-day-old shoots were placed in metallic cages covered with 1-mm plastic screen. In each of the cages six adult *D. undecimpunctata* individuals subjected to a 24 h fast to incentivize consumption of the shoots were released. The insects were kept in the cages for 15 days to verify and quantify possible damage. The injuries produced in each cladode age, and progress of the damage was monitored were recorded. To identify differences in damage by *D. undecimpuctata*, according to shoot age, a Chi squared test was performed at a probability of less than 5% (SAS, 2016).

Simulation of physical damage

This phase was conducted under semi-controlled greenhouse conditions, and in the field in a commercial plot. In the first case, we used cages similar to those described above; in these cages we placed pots with cactus pear plants that had vegetative buds 5, 9 and 13 days of age. The shoots were punctured with a previously disinfected dissection needle in their apex, gently perforating the epidermis. There were three replications of damage simulation, and an undamaged control was included. In the field, similar punctures were performed on 4-, 5-, and 7-day-old cladodes, considering seven replications and three controls.

Under both conditions, greenhouse and field, evolution of the damage was monitored every third day for two weeks, recording the beginning of malformations and assessing the severity of the damage by measuring the depth of the slit relative to the perimeter of the cladode, as well as cladode width and length. To identify differences between the damage under semi-controlled greenhouse conditions and that caused by exposure to environmental conditions in the field, by shoot age, a Chi squared test was applied at a probability of less than 5% to determine at what age the shoot is more susceptible to damage (SAS, 2016).

Identification of phytoplasmas using PCR and RFLP

In this phase of the study, 22 buds eight- to twelve-days old were collected, 12 with heart-shape symptoms and 10 asymptomatic. In all cases, the buds were collected from mother cladodes with no visible malformations at different positions within the commercial *Opuntia ficus-indica* cv. Villanueva plantation. The samples were analyzed in the Molecular Biology Laboratory of Phytopathogens of the Center for Interdisciplinary Research for Integral Regional Development, Sinaloa Unit, National Polytechnical Institute (https://www.ciidirsinaloa.ipn.mx/).

The selected shoots were kept at 4 °C until analysis. To detect phytoplasmas, the spines were removed, and a 1-g sample of tissue was taken from the areoles near the apex of the cladode. The

DNA was extracted following the CTAB protocol (Zhang *et al*., 1998), with some modifications. The CTAB buffer was used to rupture cell membranes, and chloroform was added to precipitate proteins by centrifugation, and to obtain the DNA from the aqueous part. The RNase was then added to eliminate RNA, and chloroform was again added to precipitate the residues of proteins, RNA, and lipids, to separate and recover DNA by centrifugation. Later, isopropanol was added to form a pellet of concentrated DNA, which was washed with ethanol to eliminate the residues of CTAB buffer and chloroform. The pellet was left in repose to evaporate the ethanol, then dissolved in sterile water and kept at 4 °C. The extracted DNA was used to detect the presence of phytoplasmas and identify them using nested Polymerase Chain Reaction (PCR) techniques and Restriction Fragment Length Polymorphisms (RFLP). A molecular marker of 100 to 12,000 base pairs was used.

Results and Discussion

Damage by Diabrotica undecimpunctata

There were no differences in damage caused by *D. undecimpunctata* attributable to age of the cactus pear shoots. Although a higher preference was observed for nine-day old shoots (Figure 1), there was no conclusive evidence about *Diabrotica* preference for any shoot age (Pr > ChiSq=0.818).

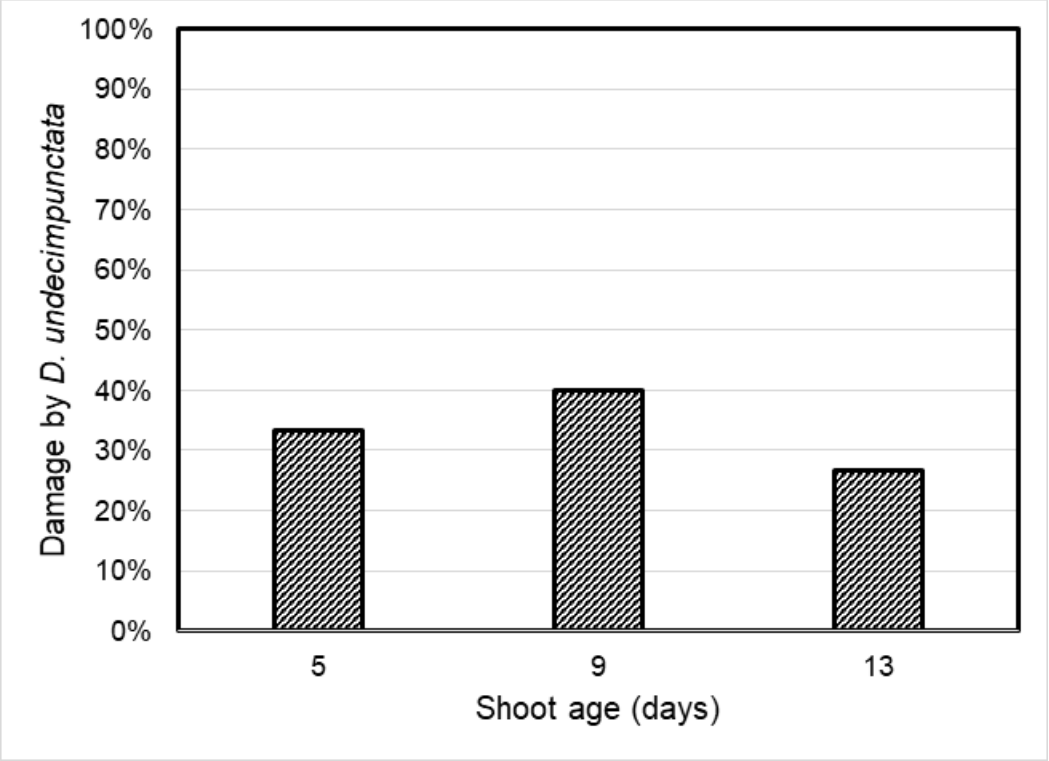

Figure 1. The incidence of damage caused by *D. undecimpunctata* feeding on cladodes of *O. ficus-indica* cv. Villanueva.

Three days after the experiment began, lesions were observed on both faces of the cladodes. The damage evolved into superficial circles 5 mm in diameter that passed through the cuticle and spongy tissue until they reached the cuticle on the opposite side of the cladode. *D. undecimpunctata* fed for nine days, and during this time the lesions took on irregular shapes and developed rough, rigid whitish scars (Figure 2).

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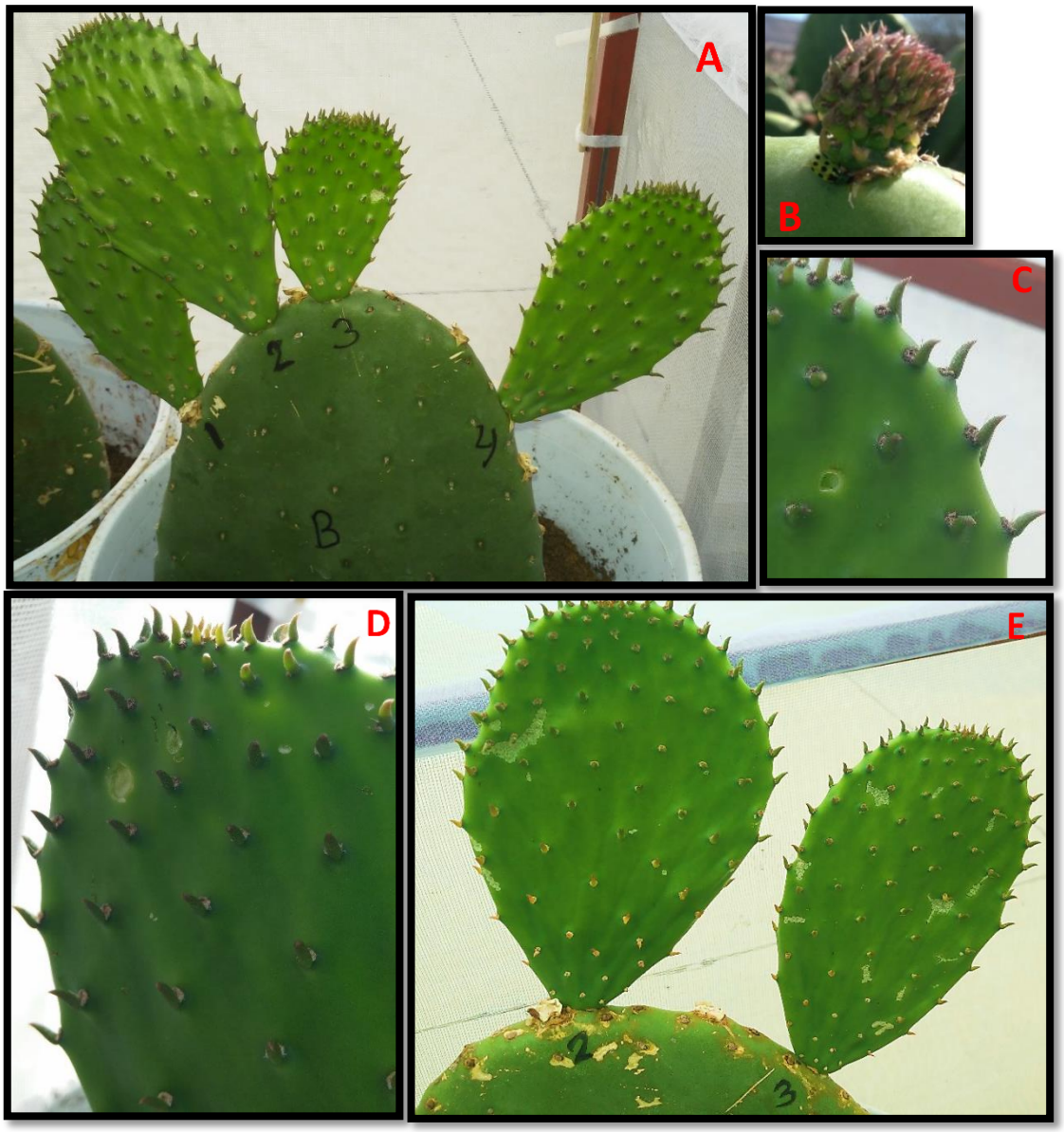

Figure 2. The damage caused by *D. undecimpunctata* on *Opuntia ficus-indica* cv. Villanueva shoots. A: damage to shoots of different ages, B: *D. undecimpunctata* feeding on a small shoot. C and D: Fresh damage from feeding *D. undecimpunctata*, E: scarring of tissue damaged by *D. undecimpunctata.*

After this time, most of the *D. undecimpunctata* individuals died; the only one remaining continued feeding on 20 and 24-day-old cladodes. The assumption that this pest feeds on shoots that are tender enough for them to tear the cuticle. Possibly, they later emigrate in search of tender shoots or to other plants since this is a generalist insect that feeds on a wide range of plant species (Graham *et al.*, 2012; Gaillard *et al*., 2018; Pereira *et al.,* 2019).

The high moisture content, as in the cactus pear cladodes, and the presence of damage by insects or other causes favor transmission of pathogenic agents between plants (Melotto *et al*., 2008). Moreover, the presence of weeds and other host plants could favor the incidence of pests and, consequently, the

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possible dissemination of pathogens (Sánchez *et al*., 1998; Franco *et al*., 2021). In the case of *D. undecimpunctata*, 33 host plants have been identified (Graham *et al*., 2012), and the damage they can cause becomes severe because it is a multivoltine species (Roberto *et al*., 2001) that can spawn several generations in a single year. Furthermore, many *Diabrotic* species feed on roots (larvae) and above ground parts (adults) of a large diversity of plants and are considered vectors of diseases that are lethal for cactus pear (Derunkov & Konstantinov, 2013). For this reason, integral phytosanitary management should be one of the priorities of commercial cactus plantations. Additionally, it cannot be ruled out that the heart-shaped cladode malformations are due to the combined effect of several factors.

Simulation of physical damage

The simulation of physical damage under semi-controlled greenhouse conditions did not cause differences (Prob>ChiSq=1.000) in heart-shape symptoms among the shoots. The percentage of cladodes affected was equal at all shoot ages (Figure 3). For the three evaluated ages, the symptomatic cladodes began to exhibit heart-shape symptoms seven days after inflicting the lesion with the needle. The resulting slit had a depth of 0.4 to 1.6 cm at all evaluated ages.

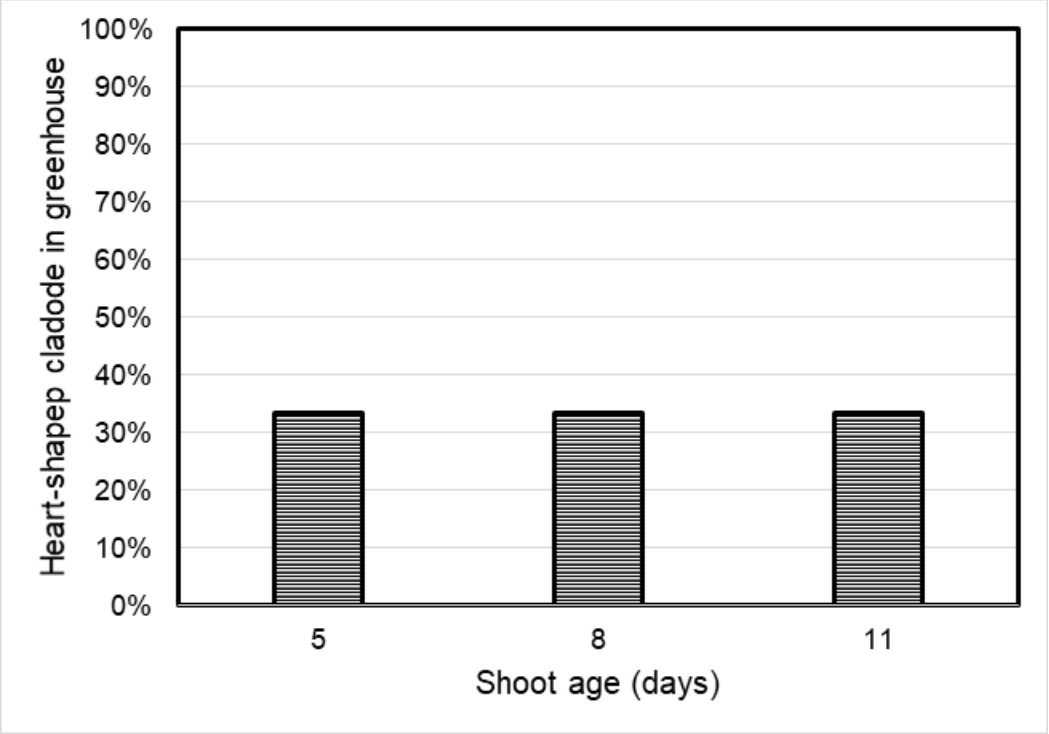

Figure 3. The incidence of heart-shaped cladode symptoms caused by physical damage to *O. ficus-indica* cv. Villanueva shoots under controlled conditions.

In the case of simulation of physical damage in field conditions within a commercial plot, the cladodes of the three ages tested showed symptoms of heart-shaped cladode as of three days after the lesion (Figure 4). The percentage of cladodes with symptoms was higher in 7-day-old cladodes (37.5%). Twenty-nine days after causing the lesion, the induced injury was deeper in cladodes that were younger in the moment of inflicting the lesion. The 4-day-old cladodes developed slits between 7.2 and 9.4 cm deep; in 5-day-old cladodes the lesions were 1.2 to 7.0 cm deep, and in the seven-day-old

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cladodes they were 1.6 to 5.3 cm deep. By comparison, the lesions were more severe in the commercial plot than under semi-controlled greenhouse conditions. This could be associated with a combined effect of the damage caused with the needle and exposure to more severe environmental conditions that change throughout the day.

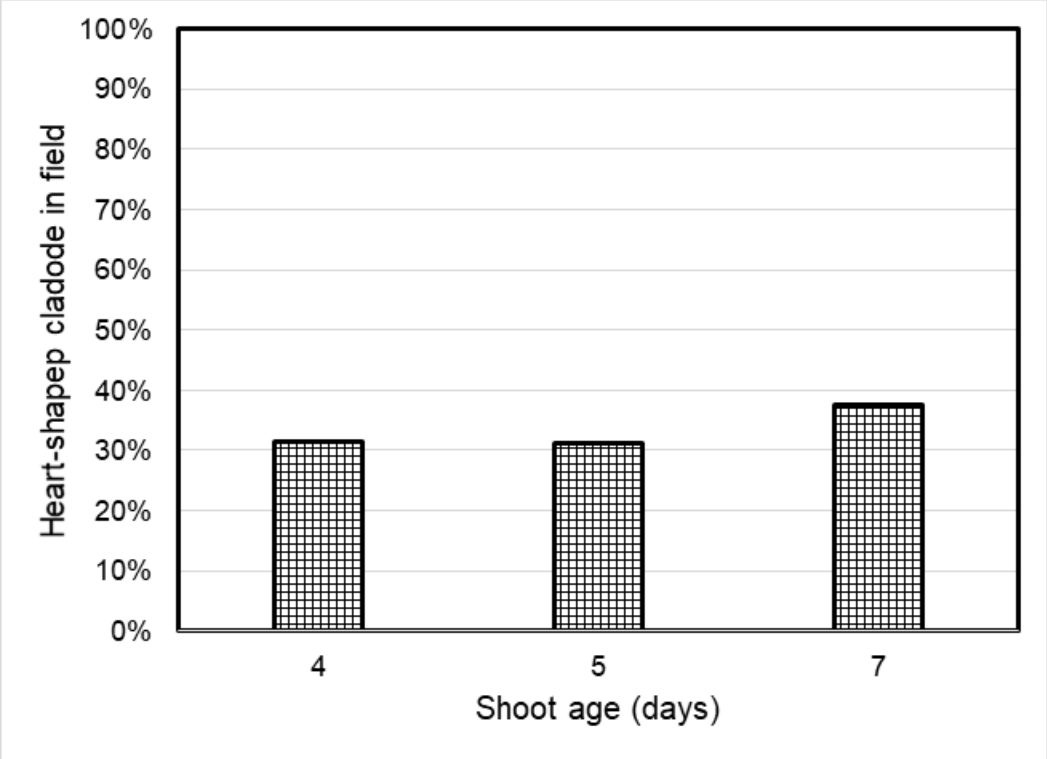

Figure 4. The incidence of heart-shaped cladode symptoms caused by physical damage to *O. ficus-indica* cv. Villanueva shoots under field conditions.

Detection of phytoplasmas using PCR

In the field, 22 shoots were obtained; however, 14 were used (eight with heart-shape symptoms and six asymptomatic) in the direct PCR amplification phase. The samples used were those that exhibited better quality and integrity of the extracted DNA, according to the concentration of nucleic acids (quality between 1.8 and 2.0). The amplification was observed in all the bands, considering the negative and positive controls. A nested PCR test was then conducted for a more specific search in the chain of base pairs.

The presence of a band between the range of 1,000 to 1,650 base pairs suggests the presence of a phytoplasma within that weight (Martínez-Salgado *et al*., 2020), evidenced by a coincidence in the bands of the samples and the positive control. The use of nested PCR test for amplification of DNA of a specific fragment of base pairs, was useful to determine three cladodes tested positive to the presence of phytoplasmas, two symptomatic and one asymptomatic. Although the presence of phytoplasmas was detected, it is possible that they are not the cause of heart-shape, or at least they are not the only factor involved, since they were detected in both symptomatic and asymptomatic cladodes.

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Identification of phytoplasmas using RFLP

The RFLP test was useful to determine that the phytoplasmas of the samples diagnosed as positive by PCR belong to the 16SrI, 16SrII and 16SrIII groups. This was determined by comparison of the amplified bands that were restricted by two enzymes, Hinf I (GANT/C and C/TNAG) and Kpn I (GGTAC/C and C/CATGG). The fragment detection was located within the 1,350 bp region (Figure 5), which agrees with results of Gundersen *et al*. (1996) for 16Sr phytoplasmas.

The phytoplasmas of the 16Srll group have been identified in *Opuntia* species; they cause the malformation known as witch's broom (Cai *et al*., 2008; Zheng-Nan *et al*., 2012). A symptom specific to this malformation is the proliferation of vegetative shoots associated to a hormonal imbalance (Costa *et al*., 2021). In addition, Reveles-Torres *et al*. (2014) indicate that the diversity of phytoplasma strains in group II can be explained in part by the presence of vectors, especially phytophagous insects. A recent report indicates the existence of 41 *Candidatus* species, 33 ribosomal groups, and 160 subgroups in a large variety of wild and cultivated plants, including cereals, fruit trees, and medicinal plants (Hemmati *et al*., 2021); others include ornamental cacti (Salar *et al*., 2007), as well as the genus *Opuntia* in Lebanon (Choueiri *et al*., 2005) and México (Aviña-Padilla *et al*., 2009). Moreover, Prasetya *et al*. (2018) identified phytoplasmas of the subgroup 16Srll-C associated with *Opuntia* sp. causing yellowing, thickening and deformation of the cladodes as well as heart-shaped shoots.

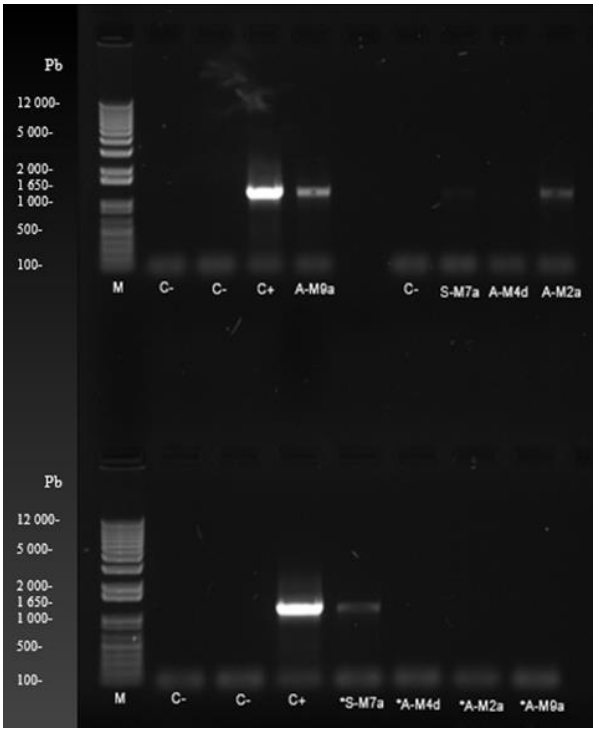

Figure 51. The nested amplification of cactus pear DNA. M= molecular weight marker, C-=negative control and C+=positive control. Sample code: S=healthy, A=heart-shaped, M=plot number (M2), lower case letters indicate the replication number (a=1, b=2, c=3, d=4, e=5, f=6).

In addition, Suaste-Dzul *et al*. (2012) determined the presence of phytoplasmas in *O. ficus-indica* cladodes with or without symptoms of deformation, as is the case of this study (Figure 6). This could have implications for propagating diseases using as seed cladodes that could contain phytoplasmas but have no visible symptoms. Fucikovsky *et al*. (2011) also identified phytoplasmas in healthy and

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diseased cladodes with the symptom of inhibition of fruit production, characteristic of cactus pear "macho plants", which also exhibit thickening and heart-shaped cladodes; these symptoms have been associated with phytoplasmas of the species *Candidatus* Phytoplasma asteris in the 16SrI group. These phytoplasmas have been also found in other plants (*Argemone mexicana* and *Lupinus* sp.), as well as in bugs (*Chelinidae* sp.), which can function as reservoirs and vectors of phytoplasmas, respectively (Fucikovsky *et al*. 2011). Therefore, it is important to control weeds and insects in areas surrounding cactus pear plantations to reduce the risk of damage by phytoplasmas, among other pathogens. It is worth mentioning that in the plantations of the present study, this type of insect and plants of the genus *Argemone* were present.

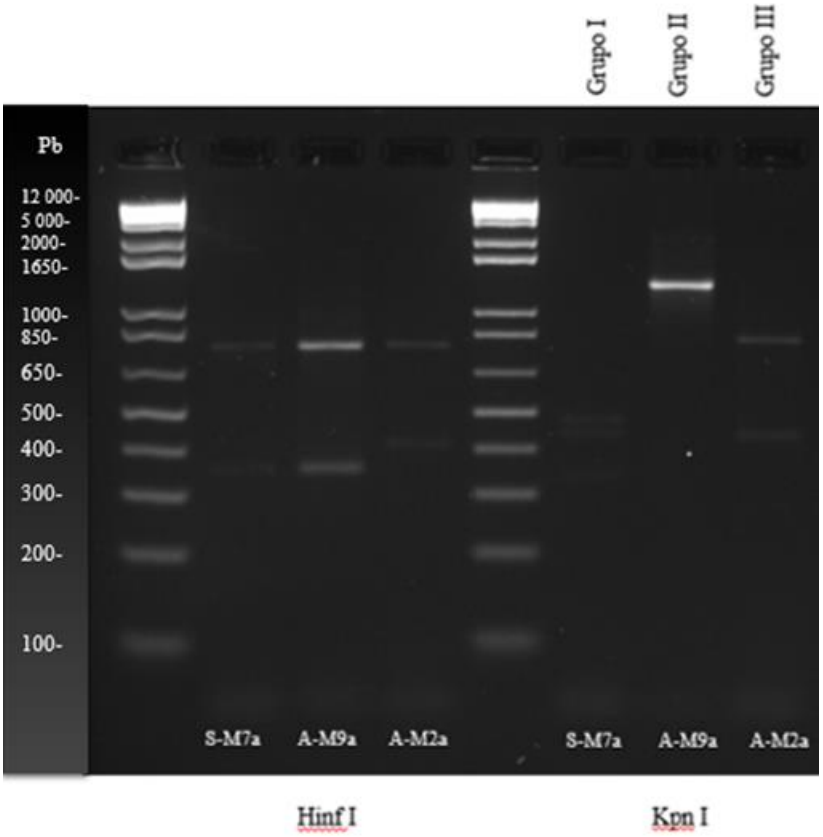

Figure 6. The DNA samples amplified with the enzymes Hinf I and Kpn I. Sample code: S= healthy, A= heart-shaped, M= plot number (M2), lowercase letters indicate replication number (a=1, b=2, c=3, d=4, e=5, f=6).

Based on the results, it is possible to consider that heart-shaped cladodes in *O. ficus-indica* is caused by a phytoplasma. However, it is not possible to assert that it is the only agent that causes this malformation given that phytoplasmas have also been recorded in healthy cladodes that were asymptomatic but tested positive. This result makes it necessary to dilucidate the possible transmission of phytoplasmas through symptomatic and asymptomatic cladodes to healthy cladodes to determine conclusively whether these microorganisms are or are not causal agents of heart-shaped cladodes. It is relevant to implement actions to prevent the eventual propagation of phytoplasmas to other plantations through insect transmitters or using apparently healthy seed cladodes.

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Conclusions

Although *Diabrótica undecimpunctata* is an insect that feeds on tender cactus pear shoots, it does not cause the heart-shape cladode malformation. Puncturing with a needle in the apical part of tender shoots caused the appearance of symptoms like those of heart-shaped cladode. The phytoplasmas were detected in both symptomatic and asymptomatic cactus pear shoots, and therefore, it is not possible to assert that they are causal agents of this type of malformation in cactus pear. Although the damage by *D. undecimpunctata* was tested, induced physical lesions, and registered the presence of phytoplasmas, it was not possible to identify the causal agent of heart-shaped cladode in *Opuntia ficus-indica*.

ETHICS STATEMENT

Ethical approval is not applicable for this article.

CONSENT FOR PUBLICATION

The author warrants that their contribution is original and has not been published before (except as part of a thesis).

COMPETING INTEREST

The authors declare that they have no competing interests.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are available and were part of a M.Sc. thesis research.

AUTHOR CONTRIBUTION

Conceptualization, M.E.M.-O., S.d.J.M.-G. Methodology, M.E.M.-O., S.d.J.M.-G., J.M.-C. Validation, S.d.J.M.-G., J.M.-C. Formal analysis, M.E.M.-O, F.J.M.-F., S.d.J.M.-G. Research, M.E.M.-O., Resources, S.d.J.M.-G., J.M.-C. Visualization, I.H.-R., S.d.J.M.-G., Literature review, M.E.M.-O., I.H.-R., Supervision, S.d.J.M.-G., I.H.-R., Review of the final version and approval of the manuscript before sending it, I.H.-R., S.d.J.M.-G.

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References

Aviña-Padilla, K., Parra-Cota, F., Ochoa-Sánchez, J.C., Perales-Segovia, C. and Martínez-Soriano, J. P. 2009. Phytoplasmas associated to diseases of ornamental cacti in México. *Journal of Biological Sciences.* 9(3):268-271. https://doi.org/10.3923/jbs.2009.268.271

Batista-Dubeux, J.C., Ferreira dos Santos, M.V., Vieira da Cunha, M., Cordeiro dos Santos, D., de Almeida Souza, R.T., Leão de Mello, A.C. and Carvalho de Souza, T. 2021. Cactus (*Opuntia* and *Nopalea*) nutritive value: A review. *Animal Feed Science and Technology*. 275:114890. https://doi.org/10.1016/j.anifeedsci.2021.114890

Cai, H., Wei, W., Davis, R.E., Chen, A. and Zhao, Y. 2008. Genetic diversity among phytoplasmas infecting *Opuntia* species: virtual RFLP analysis identifies new subgroups in the peanut witches'-broom phytoplasma group. *Journal of Systematic and Evolutionary Microbiology.* 58:1448-1457. https://doi.org/10.1099/ijs.0.65615-0

Choueiri, E., Massad, R., Jreijiri, F., Danet, J.L., Salar P., Bové J.M. and Foissac, X. 2005. First report of a 16SrII group phytoplasma associated with shoot proliferation of a cactus (*Opuntia monacantha*) in Lebanon. *Plant Disease*. 89(10):1129-1130. https://doi.org/10.1094/PD-89-1129B

Costa, J.L., Paschoal, D., da Silva E.M., Silva J. S., do Carmo, R.M., Carrera, E., López-Díaz, I., Rossi, M.L., Freschi, L., Mieczkowski, P., Peres, L., Teixeira, P.J.P.L. and Figueira, A. 2021. *Moniliophthora perniciosa*, the causal agent of witches' broom disease of cacao, interferes with cytokinin metabolism during infection of Micro-Tom tomato and promotes symptom development. *New Phytologist*. 231(1):365-381. https://doi.org/10.1111/nph.17386

Derunkov, A., and Konstantinov, A. 2013. Taxonomic changes in the genus *Diabrotica* Chevrolat (Coleoptera: Chrysomelidae: Galerucinar): results of a synopsis of North and Central America *Diabrotic* species. *Zootaxa* 3686(3):301–325. https://doi.org/10.11646/zootaxa.3686.3.1

Fontenele, R.S., Salywon, A.M., Majure, L.C., Cobb, I.N., Bhaskara, A., Avalos-Calleros, J.A., Argüello-Astorga, G.R., Schmidlin, K., Khalifeh, A., Smith, K., Schreck, J., Lund, M.C., Köhler, M., Wojciechowski, M.F., Hodgson, W.C., Puente-Martinez, R., Van Doorslaer, K., Kumari, S., Vernière, C., Filloux, D., Roumagnac, P., Lefeuvre, P., Ribeiro, S.G., Kraberger, S., Martin D.P. and Varsani, A. 2020. A novel divergent geminivirus identified in asymptomatic New World Cactaceae plants. *Viruses*. 12(4): 398. https://doi.org/10.3390/v12040398

Franco, F.P., Túler, A.C., Gallan, D.Z., Gonçalves, F.G., Favaris, A.P., Peñaflor, M.F.G.V., Leal, W. S., Moura, D.S., Bento, J.M.S. and Silva-Filho, M.C. 2021. Fungal phytopathogen modulates plant and insect responses to promote its dissemination. *The ISME Journal*. 15*(*12):3522-3533. https://doi.org/10.1038/s41396-021-01010-z

Fucikovsky, Z., Yañez-Morales, M.J., Alanís-Martínez, I. and González-Pérez, E. 2011. New hosts of 16SrI phytoplasma group associated with edible *Opuntia ficus-indica* crop and its pests in México. *African Journal of Microbiology Research*. 5(8):910-918. https://doi.org/10.5897/AJMR10.846

Gaillard, M.D.P., Glauser, G., Robert, C.A.M. and Turlings, T.C.J. 2018. Fine-tuning the 'plant domestication-reduced defense' hypothesis: specialist vs generalist herbivores. *New Phytologist.* 217:355-366. https://doi.org/10.1111/nph.14757

Graham, E., Tooker, J. and Hanks, L. 2012. Floral host plants of adult beetles in central Illinois: an historical perspective. *Annals of the Entomological Society of America*. 105(2):287-297. https://doi.org/10.1603/AN11120

Granata, G., Paltrinieri, S., Botti, S. and Bertaccini, A. 2006. Aetiology of *Opuntia ficus-indica* malformations and stunting disease. *Annals of Applied Biology.* 149:317-325. https://doi.org/10.1111/j.1744-7348.2006.00097.x

Granata, G., Faedda, R. and Ochoa, M.J. 2018. Enfermedades del nopal. In: Inglese, P., Mondragón-Jacobo, C., Nefzaoui, A. and Sáenz, C. (eds). Organización de las Naciones Unidas para la Alimentación y la Agricultura, Centro Internacional de Investigaciones Agrícolas en Zonas Áridas. Roma. 1ª Ed.120-127 ISBN 978-92-5-130494-5 (FAO).

Gundersen, D.E., Lee, I.M., Schaff, D.A., Harrison, N.A., Chang, C.J., Davis, R.E. and Kingsbury, D.T. 1996. Genomic diversity and differentiation among phytoplasma strains in 16s rRNA groups I (Aster yellows and related phytoplasmas) and I11 (X-Disease and related phytoplasmas). *International Journal of Systematic and Evolutionary Microbiology. 46*(1):64-75. https://doi.org/10.1099/00207713-46-1-64

Hernández-Pérez, R., Noa-Carrazana, J.C., Gaspar, R., Mata, P. and Flores-Estévez, N. 2009. First report of symptoms associated to a phytoplasma affecting nopal (*Opuntia ficus indica* Mill) in the "Pyramids" Indian, State of México. *Journal of General and Molecular Virology*. 1(4):046-047. https://doi.org/10.5897/JGMV.9000007

Hemmati, Ch., Nikooei, M., Al-Subhi, A.M. and Al-Sadi, A.M. 2021. History and current status of phytoplasma diseases in the Middle East. *Biology*. 10(226):1-20. https://doi.org/10.3390/biology10030226

Hernández-Sánchez, E., Mora-Aguilera, G., Tlalpal-Bolaños, B., Rodríguez-Leyva, E. and Alvarado-Rosales, D. 2014. Efecto de intensidad inicial de enfermedad en la caracterización temporal y espacial de la mancha negra del nopal (*Opuntia ficus-indica*). *Revista Mexicana de Fitopatología.* 32(2): 132-146.

Hernández-Pérez, R., Noa-Carrazana, J.C., Gaspar, R., Mata, P. and Flores-Estévez, N. 2009. Detection of phytoplasma on indian fig (*Opuntia ficus-indica* Mill) in Mexico central region. *OnLine Journal of Biological Sciences.* 9(3):62–66. https://doi.org/10.3844/ojbsci.2009.62.66

Martínez-Salgado, F., Pérez-Moreno, J., Ochoa-Martínez, D.L., Rojas-Martínez, R.I. and Arellano-Ostoa, G. 2020. Etiology of cladode thickening of prickly pear cactus (*Opuntia ficus-indica* (L.) Mill.) in Mexico. *Agrociencia.* 54(4):521-530. https://doi.org/10.47163/agrociencia.v54i4.2048

Mazzeo, G., Nucifora, S., Russo, A. and Suma, P. 2019. *Dactylopius opuntiae*, a new prickly pear cactus pest in the Mediterranean: an overview. *Entomologia Experimentalis et Applicata.* 167(1):59-72. https://doi.org/10.1111/eea.12756

Melotto, M., Underwood, W. and He, S.Y. 2008. Role of stomata in plant innate immunity and foliar bacterial diseases. *Annual Review of Phytopathology.* 46:101-122. https://doi.org/10.1146/annurev.phyto.121107.104959

Mena-Covarrubias, J. 2018. Insectos plaga del nopal. In: ecología del cultivo, manejo y usos del nopal. In: Inglese, P., Mondragón-Jacobo, C., Nefzaoui, A. and Sáenz, C. (eds). Organización de las Naciones Unidas para la Alimentación y la Agricultura, Centro Internacional de Investigaciones Agrícolas en Zonas Áridas. Roma. 1ª Ed. 129-138 pp. ISBN 978-92-5-130494-5 (FAO).

Méndez-Gallegos, S.J., Mena-Covarrubias, J., Gallegos-Vázquez, C. and Mondragón-Jacobo, C. 2013. Principales enfermedades y recomendaciones para su control en el nopal tunero. *In*: Gallegos V.C., Méndez, G.S.J. and Mondragón, J. (eds). Producción sustentable de la tuna en San Luis Potosí. Colegio de Postgraduados. Fundación Produce San Luis Potosí. San Luis Potosí, SLP. México. pp. 163-172.

Mendoza-Orozco, M.E., Hernández-Ríos, I., Morales-Flores, F.J., Mena-Covarrubias, J., Ortega-Espinoza, J., Mondragón-Jacobo, C. and Méndez-Gallegos, S.J. 2018. Heart-shaped cladodes in commercial cactus pear plantations. *Journal of the Professional Association for Cactus Development.* 20:52-67.

Moran, V.C. 1980. Interactions between phytophagous insects and their *Opuntia* hosts. *Ecological Entomology*. 5(2):153-164. https://doi.org/10.1111/j.1365-2311.1980.tb01136.x

Nefzaoui, A. 2018. *Opuntia ficus-indica* productivity and ecosystem services in arid areas. *Italus Hortus* 25(3):29-39. https://doi.org/10.26353/j.itahort/2018.3.2939

Ochoa, M.J., Lobos, E., Portillo, L. and Vigueras, A.L. 2015a. Importance of biotic factors and impact on cactus pear production systems. VIII International Congress on *Cactus Pear* and Cochineal. *Acta Horticulturae.* 1067. International Society for Horticultural Science. https://doi.org/10.17660/ActaHortic.2015.1067.45

Ochoa, M.J., Rivera, L.A., Arteaga-Garibay, R.I., Martínez-Peña, M.D., Ireta, J. and Portillo, L. 2015b. Black spot caused by *Pseudocercospora opuntiae* in cactus pear productive systems of Jalisco, Mexico. *Journal of the Professional Association for Cactus Development*. 17: 1-12.

Omar, A.F. and Foissac, X. 2012. Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. *European Journal of Plant Pathology.* 133:353-360. https://doi.org/10.1007/s10658-011-9908-x

Pemberton, R.W. and Cordo, H.A. 2001. Potential and risks of biological control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. *Florida Entomologist Society*. 84(4):513-526. https://doi.org/10.2307/3496382

Pereira, A.E., Tenhumberg, B., Meinke, L.J. and Siegfried, B.D. 2019. Southern corn rootworm (Coleoptera: Chrysomelidae) adult emergence and population growth assessment after selection with vacuolar ATPase-A double-stranded RNA over multiple generations. *Journal of Economic Entomology*. 112(3):1354-1364. https://doi.org/10.1093/jee/toz008

Prasetya, A., Mutaqin, K.H., Sinaga, M.S. and Giyanto, G. 2018. Diversity of phytoplasmas associated with several plants in Western Java-Indonesia. *Walailak Journal of Science and Technology.* 17(4):303-312. https://doi.org/10.48048/wjst.2020.3939

Reveles-Torres, L.R., Velásquez-Valle, R. and Mauricio-Castillo, J.A. 2014. Fitoplasmas: Otros agentes fitopatógenos. Folleto Técnico 56. Campo Experimental Zacatecas. CIRNOC-INIFAP. 41 p.

Roberto, S.R., Genta W. and Ventura, M.U. 2001. *Diabrotica speciosa* (Ger.) (Coleoptera: Chrysomelidae): New pest in table grape orchards. *Neotropical Entomology*. 30(4):721-722. https://doi.org/10.1590/S1519-566X2001000400030

Salar, P., Choueiri, E., Jreijiri, F., El Zammar, S., Ean-Luc, D. and Foissac, X. 2007. Phytoplasmas in Lebanon: characterization of *Candidatus* Phytoplasma pyri' and stolbur phytoplasma respectively associated with pear decline and grapevine "bois noir" diseases. *Bulletin of Insectology.* 60(2):357-358.

Sánchez, M.V., Agüero, R. and Challiette, R. 1998. Plantas hospederas de los virus más importantes que infectan el melón, *Cucumis melo* (Cucurbitaceae) en Costa Rica. *Revista de Biología Tropical.* 46:13-25. https://doi.org/10.15517/rbt.v46i1.19348

SAS. 2016. Base SAS® 9.4 Procedures Guide: Statistical Procedures, Sixth Edition. SAS Institute Inc. Cary, NC: SAS Institute Inc.

Singh, S., Dey, S.S., Bhatia, R., Batley, J. and Kumar, R. 2018. Molecular breeding for resistance to black rot [*Xanthomonas campestris* pv. *campestris* (Pammel) Dowson] in Brassicas: recent advances. *Euphytica.* 214:196:1-17. https://doi.org/10.1007/s10681-018-2275-3

Spallino, R.E., Rizza, S., Oliveri, C., Catara, V., Marzachi, C. and Tessitori, M. 2017. Plant teratologies as a result of phytoplasma infections. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology.* 151(5):931-939. https://doi.org/10.1080/11263504.2017.1330779

Suaste-Dzul, A., Rojas-Martínez, R.I., Zavaleta-Mejía, E. and Pérez-Brito, D. 2012. Detección molecular de fitoplasmas en nopal tunero (*Opuntia ficus-indica*) con síntomas de engrosamiento del cladodio. *Revista Mexicana de Fitopatología*. 30(1):71-80.

Swart, W. and Swart, V. 2003. An overview of research on diseases of cactus pear in South Africa. *Journal of the Professional Association for Cactus Development.* 05:115-120.

Zacarías-Alvarado, J.R., Tovar-Robles, C.L., Méndez-Gallegos, S.J., Magallanes-Quintanar, R. and Aquino-Pérez, G. 2020. Yield and quality of carminic acid of *Dactylopius coccus* Costa in different height levels of the nopaloteca. *Agrociencia.* 54(5):705-716. https://doi.org/10.47163/agrociencia.v54i5.2127

Zhang, D., Ghislain, M., Huamán, Z., Golmirzaie, A. and Hijmans, R. 1998. RAPD variation in sweetpotato (*Ipomoea batatas* (L.) Lam) cultivars from South America and Papua New Guinea. *Genetic Resources and Crop Evolution.* 45:271–277. https://doi.org/10.1023/A:1008642707580

Zheng-Nan, L., Zhang, L., Liu, P., Yao-Bo, B., Xiao-Gang, Y. and Yun-Feng, W. 2012. Detection and molecular characterization of cactus witches'-broom disease associated with a group 16SrII phytoplasma in northern areas in China. *Tropical Plant Pathology.* 37(3):210-214. https://doi.org/10.1590/S1982-56762012000300008

Zimmermann, H.G. and Granata, G. 2002. Insect pests and diseases. In: Cacti. Biology and uses. Nobel, P.S. (ed.). University of California Press, Ltd., London, England. pp. 235-254. Print ISBN-10: 0-520-23157-0.