Heart-shaped cladodes in commercial cactus pear plantations

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Received: November 7, 2017; Accepted: April 9, 2018

ABSTRACT

Because of the diverse uses it has, and the multiple benefits it contributes to, cactus pear (Opuntia spp.) is considered one of the natural resources of greatest socioeconomic importance in Mexico. Its intensive production in monoculture, associated with a reduced genetic variability, has resulted in the proliferation of various physiopathies, and the prevalence of pests and diseases, which reduce its productivity. In diverse cactus pear producing zones in Mexico and other countries, a malformation called in this study cladode 'acorazonamiento' (heart-shaped cladode), which is derived from the shape the cladode takes, has been observed; however, the causal agent is unknown at the time. Considering that its occurrence could cause economic losses to cactus pear producers, this study describes the visual symptomatology and the incidence and severity of heart-shaped cladode symptoms in commercial cactus pear plantations in the south and southeast of Zacatecas, Mexico. The symptomatology was more evident in tender buds of 6 to 20 days of age, and is characterized by the presence of diverse malformations in the apical or lateral part of the cladodes, as well as by the appearance of micro-scarring that reduces the vegetative and floral budding in the affected zone; the damaged tissue presents rupture of the cuticle and epidermis, and the detachment of cell walls. Three principal malformation symptoms were found, which were named in this study as: 'heart', 'bean' and 'saw', according to the cladode physical appearance, with the first one being the most frequent. All the plantations evaluated showed the presence of malformed cladodes, with values that fluctuated between 12 and 30 %. Cladodes with heartshape symptomatology may generate buds with similar appearance in a proportion of 2:1 asymptomatic:symptomatic cladodes. Likewise, it was observed that the degree of severity ranged between 1 to 38% of photosynthetic area reduction, and damaged cladodes may lose between 50 to 80% of vegetative and floral buds. Derived from this, it is pertinent to perform

JPACD 2018 (20): 52-67

studies directed towards characterizing its etiology in order to implement management strategies and reduce economical losses.

Keywords: *Opuntia*, physiopathies, etiology, diseases, productivity.

INTRODUCTION

In Mexico, cactus pear (*Opuntia* spp) is considered one of the most important plant resources because there is an ancient tradition on the consumption of its fruits and edible tender cladodes (Zheng-Nan *et al.*, 2012; Dewir *et al.*, 2015; López-Gutiérrez *et al.*, 2015;). In just a few decades, it has evolved from an informal to a commercial crop for the production of fodder, fruit, and natural coloring for the market (Granata and Sidoti, 2002). In Mexico, the land cultivated with *Opuntia* for nopalitos (edible tender cladodes) comprises 12,595 ha, while 47,984 ha are used for fruit production (SIAP, 2017).

Cactus pear plantations are affected by pests (De Jesús *et al.*, 2016), biotic or infective diseases (bacteria, mycoplasmas, fungi, nematodes and phytoplasms, among others), and abiotic or non-infective illnesses caused by adverse climate conditions, nutritional deficiencies, genetic abnormalities, improper crop management, and incorrect application of agrichemicals, among others (Granata, 1995; Granata and Sidoti, 2002), these factors limit this crop productivity, or even lead to total losses (Quezada-Salinas *et al.*, 2006).

Particularly in cactus pear, the interaction between phytopathogens and abiotic factors cause alterations that can mislead the symptomatology and complicate the identification of the causal agents. It is inferred that some environmental factors, as well as the particular physiology of this species, can stimulate the development of diseases in cactus pear (Swart and Swart, 2003). On the other hand, the perennial character of the infected tissue, and pruned cladodes that remains on the ground, worsens the susceptibility of cactus pear (Hernández-Sánchez *et al.*, 2014). Due to this, to avoid the dispersion of diseases, it is necessary to exert a strict control in the transport of propagation material or its import from other countries (Granata and Sidoti, 2002).

Recently, cladodes with the presence of apical or lateral deformations have been observed in both wild populations and commercial cactus pear plantations, whose symptoms are encompassed in this study under the term cladode 'acorazonamiento' (heart-shaped cladodes). Its presence causes the reduction of the cladode photosynthetic area, and a low budbreak of floral and vegetative buds because the symptoms are presented primarily in the cladode's growth zone, where a high density of buds is located; therefore, production and quality of both fruit and nopalitos are reduced. It has to be mentioned that these deformations are not consistent with the symptomatology found in previous studies on phytoplasmas (Osorio *et al.*, 1997; Granata *et al.*, 2006; Hernández-Pérez *et al.*, 2009; Fucikovsky *et al.*, 2011; Suaste *et al.*, 2012; Dewir *et al.*, 2015). An important aspect to consider in designing a management strategy is to quantify the intensity of the damage and its risk of occurrence. Considering this,

the study characterizes the symptoms associated to the malformation, the potential economic losses are estimated, and the incidence and severity of cladode 'acorazonamiento' is determined in commercial cactus pear plantations, in order to contribute to reducing the vulnerability and productive instability of the cactus pear cultivation system.

MATERIALS AND METHODS

Study area

The study was performed during 2015-2016, in eight commercial cactus pear plantations: two for fruit production and six for nopalitos production, located in the municipalities of Pinos and Villanueva, in Zacatecas, Mexico. These zones are considered as highly important because of the extended land areas used for either fruit or nopalitos production, respectively. Pinos is located in the southeast of the state of Zacatecas (21°17′55" North Latitude and 101° 34′ 30" West Longitude), at an altitude of 2,300 m.a.s.l. and it is the municipality with the largest land area (7,434 ha) planted with cactus for fruit production (SIAP, 2017); the annual mean temperature is 16°C, and the average precipitation is 480 mm. On the other hand, the municipality of Villanueva is located in the south of the state (22°21′13" North Latitude and 102° 52′ 59" West Longitude), at an altitude of 1,840 m.a.s.l. The region presents a mean temperature of 18 °C, and a mean annual precipitation of 650 mm.

Characterization of the symptomatology

The visual symptoms were identified and described in cladodes having ages from 5 days to 6 months, in two commercial plantations of cactus pear cultivars in Pinos: 'Rojo Pelón' (*Opuntia ficus-indica* (L.) Mill.) and 'Cristalina' (*Opuntia albicarpa* Scheinvar); as well as, in a plantation of the cultivar 'Villanueva' (*Opuntia ficus-indica* (L.) Mill.) used for the production of nopalitos and fodder, in Villanueva. These plantations were selected considering their accessibility, owner willingness, and occurrence of damage. For each cultivar (Rojo pelón, Cristalina, and Villanueva), 100 plants and 340 cladodes were sampled (at least 3 cladodes per plant), recording the level of damage in each cladode.

To determine the morphological alterations, histological cuts were carried out in two cladodes of *O. ficus-indica cv.* Villanueva: one asymptomatic cladode (with no evident symptoms of damage), and a symptomatic one (showing evident symptoms of damage). For this purpose, spines were first removed and then 1-mm-thick histological cuts were made (frontal, transverse, and sagittal planes) using a razor blade. Later, cuts were fixed (in sodium hypochlorite at 20 % for 40 min; they were changed then to ethylic alcohol at 95% for 24 h, and thereafter to a fixing solution FAA-50 mL of ethylic alcohol at 95% + 10 mL of formic aldehyde at 40% + 2 mL of pure acetic acid + 38 mL of distilled water, remaining there for 72 h). The cuts were separated in glass jars labelled for each solution, dyed with cotton blue 0.5% and mounted on microscope glass slides with glycerin. Finally, a coverslip was placed on each preparation and sealed with transparent nail polish, having a total of 80 samples (32 asymptomatic and 48 symptomatic).

Mounted tissues were observed in an optical microscope (Leica DM 4000 B-M), and photographs were taken (Leica Application Software X Core) to describe the symptomatology of the damaged zone.

Incidence and severity

To estimate the occurrence of heart-shaped cladodes, eight commercial cactus pear plantations of different ages were sampled in Pinos and Villanueva, Zacatecas, Mexico. The proportion of damage was evaluated, registering the number of symptomatic cladodes (encompassed all as heart-shaped) over the total number of cladodes, thus obtaining the proportion of healthy or symptomatic/asymptomatic cladodes per plant (Figure 1). Likewise, the proportion of healthy, symptomatic, or both, buds originated from either symptomatic or asymptomatic mother cladodes, was determined (Figure 2) in a sample of 400 plants (reliability level of 90%). The presence of each symptom associated to the malformation was estimated in a single commercial plantation located in Villanueva, Zacatecas.

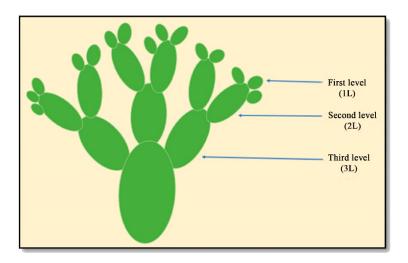


Figure 1. Graphic representation of the levels studied in cactus pear plants (1L and 2L).

The effect and severity of the damage was assessed through an estimation of the photosynthetic area reduction and the number of buds lost. Thus, 160 heart-shaped cladodes at productive age (6 to 12 months) were evaluated in a single plantation in Villanueva, Zacatecas. Photosynthetic area reduction due to damages attributed to this malformation was calculated by subtracting the missing segment from the total area within the ellipse formed by the continuous perimeter of a complete cladode.

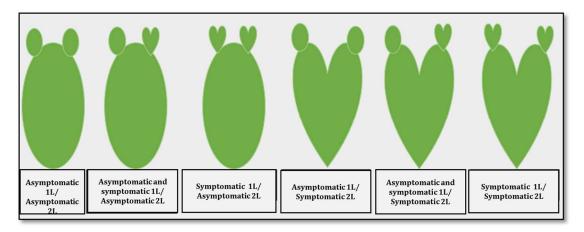


Figure 2. Combinations observed in the production of asymptomatic (healthy) or symptomatic buds, originated from asymptomatic or symptomatic mother cladodes.

To estimate the loss of buds, the average number of buds present in the central 15 cm of the apical part of asymptomatic adult cladodes was registered. Then, the proportion of bud loss was estimated from the photosynthetic area (cm²) lost due to the damage, considering the following five levels: Very low (0.77-4 %), Low (4-9 %), Medium (9-14 %), High (14-19 %) and Very high (>19 %), quantifying the percentage of cladodes found in each level evaluated (Figure 3).

The differences between symptomatic and asymptomatic cladodes were determined, as well as those for the levels of damage (t test of α =0.05); also, descriptive statistics were obtained for the characteristics of 'heart-shaped' cladodes. These tests were done with MS Excel® software.

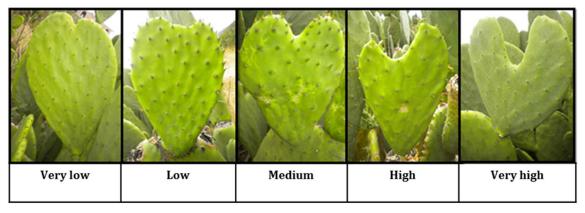


Figure 3. Levels of severity of the 'heart-shaped' cladodes in function of the loss of photosynthetic area (cm²).

RESULTS AND DISCUSSION

Symptomatology of cladode 'acorazonamiento'

Three types of cladode malformations were observed in the cactus pear plantations evaluated, which were named in this study as: "heart", "saw" and "bean", considering the shape that affected cladodes acquired (Figure 4). A well-defined linear scar was identified (1 to 3 mm width) in the damaged zone (Figure 5); likewise, an absence of budding was observed in the affected zone of symptomatic cladodes at productive age. The affected zone did not present a normal growth, since the ends in both sides of the damage keep growing, with which the invagination increases in the cladode. The cactus pad is a dicot stem with the uppermost point possessing the dominant apical bud. In other dicots such as shrubs and trees, if the apical meristem is damaged the lateral buds become dominant and grow. Something like this seems to be happening in cactus cladodes, in which the damage to the apical meristem could stimulate the lateral meristems to become dominant and the pad keeps growing, thus giving rise to a heart shape. Malformations can be observed in 5-day vegetative shoots; however, symptoms begin to be more noticeable after 6 to 20 days, when cladodes are 2 to 16 cm long.

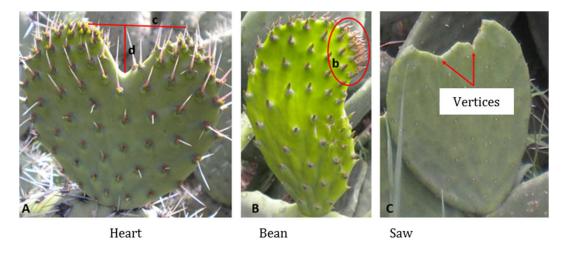


Figure 4. Detail of the types of cladode malformations observed in the field. A: Width (c) and depth (d) of the invagination. B: Lateral damage, with the apical zone displaced in the direction of the lesion. C: Vertices of the invaginations.

This malformation has been observed in both, wild cactus pear (*Opuntia streptacantha* Lem., *O. robusta* H.L. Wendl. ex Pfeiff, and *O. leucotricha* D.C.), and commercial plantations and for either, fruit (Rojo Pelón, Pico Chulo, Amarilla Plátano, Cristalina, Burrona), nopalitos (edible tender cactus like Milpa Alta) or fodder production (Atlixco, Milpa Alta, COPENA VI, COPENA FI and Villanueva, among others) including xoconostle (*O. joconostle* Web.) (Mendoza-Orozco *et al.*, 2013). However, the presence of damage is more evident in plantations used for

JPACD 2018 (20): 52-67

nopalitos production. At an international level, this malformation has also been detected in other countries, like Italy, Japan, Spain, Chile, and Peru.





Figure 5. Presence of scarring of cactus pear on the damaged part.

In Figure 6A it is shown a longitudinal cut of the damaged zone, where a light brown color scar (cz), and the presence of affected cells in cuticle (c), epidermis (e), and collenchyma, are observed. The detachment of the scar (dc), the rupture of cells caused by the lesion (Figure 6A1), cells that have suffered a rupture (Rt), and the presence of the scar (Figure 6B), are accentuated. Also, the asymptomatic tissue adjacent to the damaged zone is observed, where the scar (cz), the irregular conformation of cells in the damaged part (Figure 7C), the scarring (cz) and a group of druse crystals are observed; the vascular system is highlighted (sv) (Figure 6D).

It should be mentioned that in all the microscope glass slides observed, the vascular system adjusted towards the lateral zones parallel to the end zones of the scars was seen. It is possible to observe also the damage caused in cuticle, epidermis, and collenchyma under the chlorenchyma, as well as the rupture of cells and their detachment (Figure 7F). In Figure 7F, a hypha found in a single glass slide is seen, no mycelium or spores were observed, so the damaged cladodes could be more susceptible to the entrance of other microorganisms.

Likewise, in a single slide a set of cells arranged in a circle that could correspond to a druse of calcium crystals were seen (Figure 7G). Therefore, the 'acorazonamiento' caused by a lesion on the apical part of the cladodes affects the tissue of the cuticle, epidermis and chollenchyma. Histopathological studies with symptoms of *Pseudocercospora opuntiae* showed similar damage (Quezada *et al.*, 2013).

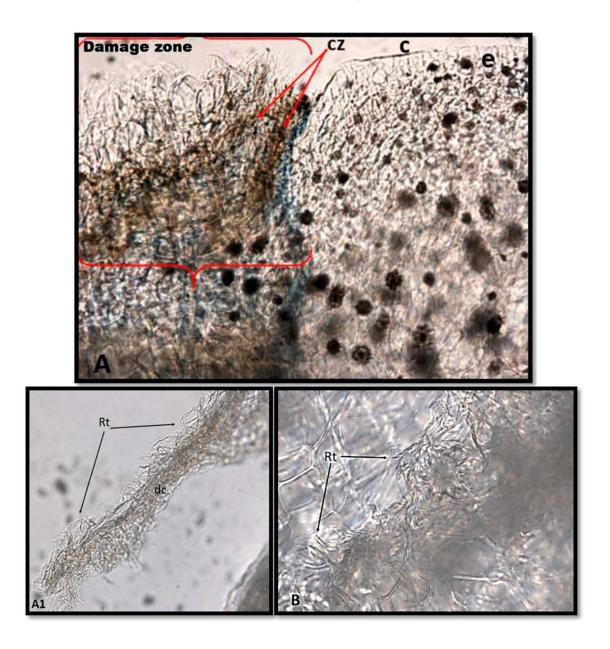


Figure 6. Longitudinal cut of the damaged zone of a heart-shaped cladode of *Opuntia ficusindica* cv. Villanue. A: damaged zone ($10x=200~\mu m$), scar (cz), cuticle (c), epidermis (e). A1: detachment of the scar (dc) ($20x=100~\mu m$). B: Rupture of the tissue (Rt) ($40x=50~\mu m$).

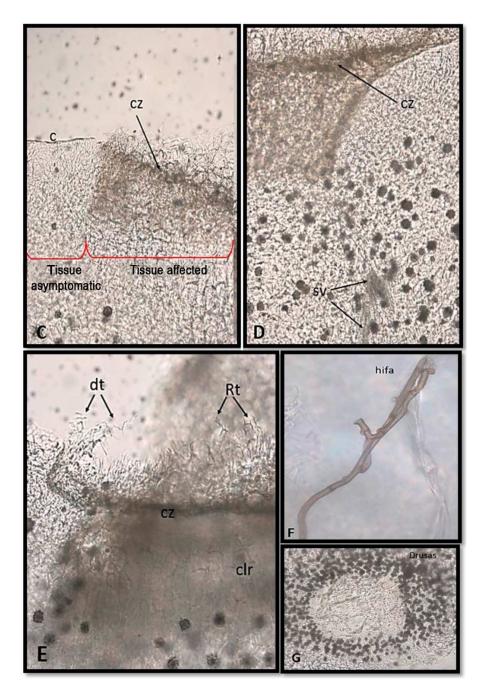


Figure 7. Structure of the tissue affected in a heart-shaped cladode of *Opuntia ficus-indica* cv. Villanueva. C and D: transversal cut ($10x = 200 \mu m$), comparison of asymptomatic and damaged tissue, cuticle (c), scar of the lesion affecting the cuticle, epidermis and chollenchyma (cz), vascular system (sv). E: longitudinal cut (10x), detachment of tissue (dt), rupture of tissue (Rt), chlorenchyma (clr), scar of the lesion (cz). F and G: parallel cut, detached hypha from the damaged zone ($40x = 50 \mu m$), group of druse crystals forming a circle (10x).

The presence of malformed cladodes has commonly been attributed to viruses and phytoplasmas (Pimienta, 1990; Granata, 1995; Osorio *et al.*, 1997; Granata and Sidoti, 2002; Fucikovsky and Yañez, 2006; Hernández-Pérez *et al.*, 2009; Fucikovsky *et al.*, 2011; Suaste *et al.*, 2012); however, they are commonly associated to yellowing, mosaic, stunting, budding on the flat part of the cladode, deformed buds, and, especially, cladode thickening. These symptoms are not consistent with those observed in this study. For example, in this research, heart-shaped cladodes were observed which can produce asymptomatic buds. Considering that the symptomatology referred here can be the product of the joint infection from virus and phytoplasmas, the diagnosis based solely on symptoms can be imprecise (Suaste *et al.*, 2012); or else, it is possible that two groups of phytoplasmas could cause the same symptom, since this consortium could express different symptomatology in the epidemiological process (Fucikovsky *et al.*, 2011).

Also, the symptomatology derived from a possible interaction between some phytopathogens and plant nutrition status could complicate the identification of the causal agent (Quezada-Salinas *et al.*, 2006). The latter is even more complicated if we take into account that several phytopathogens are associated to insects and weeds which can serve as their hosts (Fucikovsky *et al.*, 2011), facilitating disease dispersal towards healthy plants (Swart and Swart, 2003). Because of that, it is recommended to reduce the risk of this disease occurrence implementing a correct phytosanitary management, including the elimination of those cladodes with heart-shaped symptoms, while its impact is quantified and the causal agent identified (Hernández-Sánchez *et al.*, 2014).

This is particularly important since many diseases have been quickly dispersed due to the singular cell content of cactus pear, which favors the requirements of many biotic agents (Granata and Sidoti, 2002). An important aspect to consider is that some species of cactus can be immune, asymptomatic, show few symptoms, or even mask them, so that phytoplasmas may be present in both asymptomatic and sick plants (Hernández-Pérez *et al.*, 2009; Fucikovsky *et al.*, 2011).

Likewise, virus or phytoplasmas could be associated in the symptomatology, given that some cactuses are considered important hosts of phytoplasmas (Dewir *et al.*, 2015). Granata *et al.* (2006) highlight that due to the influence of environment on the dispersal of diseases, it is important to pay special attention to the environmental conditions, given that the ecological fragility of the system could place the biodiversity at risk of an epidemic outbreak of phytoplasmas. The scarce knowledge on phytoplasmas requires new studies and research in order to understand the physiological and molecular mechanisms of infection, and from an epidemiological point of view, it is important to determine the relationship between cactuses and other succulent plants with phytoplasmas and pathogens of other important crops (Dewir, 2016).

Incidence cladode 'acorazonamiento' in commercial cactus pear plantations

There were differences in the incidence of heart-shaped cladodes in the plantations of study (Table 1). The results indicated that the plantations evaluated showed the presence of plants with this malformation. The highest incidence was found in the 'Villanueva' cultivar ("Laguna" plot) with 29.8%, while the lowest was seen in the "Herbacea" plantation (12.2%) of 'Cristalina' cultivar. No systematic studies on cactus pear diseases have been conducted (Granata and Sidoti, 2002), and studies focused on disease dissemination across the main cactus producing areas in México have not been carried out either (Hernández-Pérez et al., 2009).

For *Pseudocercospora opuntiae*, Hernández-Sánchez *et al.* (2014) found that its incidence varied both regionally and seasonally, with values between 85 to 88%. For *Potexovirus*, Alonso-Barrera *et al.* (2015) registered an incidence of 47 to 60% among a series of production areas evaluated, emphasizing that dissemination occurs mainly along (rather than across) the cactus plantation lines within the plots. In the case of heart-shaped cladodes in this study, their presence in all the studied plots indicates a quick incidence expansion. However, since no documented information about this malformation is available, it can be inferred from our results that differences on its incidence are somehow related to a differential susceptibility among cultivars used for the production of either nopalitos or fruit, the agronomic management applied, or the prevailing agroclimatic conditions.

Thus, further studies could be focused on identifying the causal agent, infection routes, disease distribution in other cactus pear producing areas, species or cultivars, plant phenology, and their relationships with the sanitary management. This is especially important since cactus products are mostly fresh-consumed (Flores-Flores *et al.*, 2013), and because of the quick expansion of this crop in many countries.

Table 1. Differences in incidence (%) of heart-shaped cladodes in the cactus pear plantations evaluated.

Plot name	Casca- belito	Hormi- guero	Caballo blanco	Semi- llero	Herbacea	Central	Inverna- dero	Laguna
Cascabelito	0							
Hormiguero	0.167	0						
Caballo blanco	0.289	0.031*	0					
Semillero	0.006*	0.006*	0.000*	0				
Herbacea	0.148	0.014*	0.208	0.000*	0			
Central	0.254	0.018*	0.339	0.000*	0.371	0		
Invernadero	0.004*	0.017*	0.001*	0.434	0.001*	0.002*	0	
Laguna	0.005*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0

Note: * indicates the differences in incidence of heart-shaped cladodes presence (α =0.05)

JPACD 2018 (20): 52-67

The commercialization of nopalitos showing this kind of malformations is complicated due to their appearance and because consumers associate the damage to a bad taste of the product, causing a reduction in its price, which, according to retailers, can decrease up to 10 to 20%. The estimations attest that heart-shape malformation can cause economic losses of over 1,000 US\$ ha⁻¹, if a sales price of 0.15 US\$ per cladode is considered when marketed as propagation material for the establishment of new plantations (Table 2).

Incidence of cladode 'acorazonamiento' per level of plant and cladodes

Differences were found in the incidence between the types of malformations observed. From the total of plants sampled (581), 58% (339) of them did not present malformed cladodes on the first level of the plant, and there were quite similar values of plants with malformed cladodes on the second level (51%). With regards to cladodes in the second level, 59% were asymptomatic and 41% presented malformation symptoms, which implies that the most recent generation of cladodes are heart-shaped. However, the number of asymptomatic plants was 16% higher than those presenting some malformation. This indicates that budding with this symptomatology remains constant. In every case, the predominant symptomatology was the so called "heart" shape, with values that ranged between 24 and 33%.

Table 2. Incidence and economic impact from heart-shaped cladodes presence in commercial plantations.

N°	Name of the plantation	coord	raphic linates M-13)	Cultivar	Age of the plantation (years)	Total melgas	% incidence	Estimation of plants per plantation	Economic impact (sale 0.15 US \$/ cladode)
1	Cascabelito	715003	2472694	Villanueva	8	10	14.67	15,000	6,600
'	Cascabello	713003	2472034		O	10	14.07	13,000	0,000
2	Hormiguero	714984	2472770	Villanueva	8	11	17.22	16,500	8,525
3	Caballo blanco	716682	2471498	Villanueva	6	17	13.33	25,500	10,200
4	Semillero	710205	2463993	Villanueva	10	18	16.88	27,000	13,669
5	Herbácea	710172	2464094	Cristalina	5	17	12.22	625	2,292
6	Central	709907	2464179	Rojo Pelón	8	15	12.67	625	2,376
7	Invernadero	716765	2471531	Villanueva	10	11	23.58	16,500	11,672
8	Laguna	717022	2470456	Villanueva	3	17	29.80	25,500	22,803

Proportion of asymptomatic and heart-shaped cladodes originated from asymptomatic or heart-shaped mother stalks, in a commercial plantation of *Opuntia ficus-indica cv*. Villanueva

There were differences in the production of asymptomatic buds from either asymptomatic or heart-shaped mother stalks; however, no differences were observed in the generation of heart-

shaped buds from both types of mother stalks (Table 3). It is important to highlight that the presence of cladodes at reproductive age showing this malformation may have negative repercussions in the production of new symptomatic buds; so, it would be advisable to eliminate hearth-shaped cladodes.

The proportion of asymptomatic and heart-shaped buds from an asymptomatic mother cladode indicates that for each 3.18 asymptomatic buds there are 1.67 (0.52) heart-shaped buds. On the other hand, the proportion of asymptomatic and heart-shaped buds from a heart-shaped mother stalk is 1 and 0.62, respectively. The relation of asymptomatic and heart-shaped buds in an asymptomatic mother cladode is 1 and 0.42. The combination of asymptomatic and heart-shaped buds in the same heart-shaped mother stalk is 1 and 0.45 heart-shaped cladodes, respectively.

Table 3. Difference in the proportions of asymptomatic or heart-shaped buds from either an asymptomatic or a heart-shaped mother stalk in the first (1L) or second (2L) levels.

Variable	Media	P<0.05
Asymptomatic 1L / Asymptomatic 2L	3.18	
Asymptomatic 1L / Symtomatic 2L	2.71	0.005
* Symptomatic 1L / Asymptomatic 2L	1.12	0.390
* Symptomatic 1L / Symptomatic 2L	1.15	0.530
* Asymptomatic 1L / Asymptomatic 2L	2.66	0.376
* Asymptomatic 1L / Symptomatic 2L	2.56	0.376
Symptomatic 1L / Asymptomatic 2L	1.67	0.500
Symptomatic 1L / Symptomatic 2L	1.67	0.300

^{*}Comparisons when the proportions come from the combination between new asymptomatic and symptomatic (heart-shaped) buds in the same asymptomatic or symptomatic stalk.

Levels of severity of cladode 'acorazonamiento' symptoms

Cladodes with heart-shaped symptoms showed an average length of 29.8 cm and width of 19.6 cm, while asymptomatic ones presented a length of 41.5 cm and 23 cm width. In both types of cladodes, eccentricity value tended to 1 due to their tendency to the elliptical shape, which influences the proportion of photosynthetic area lost as a result of the deformation described here, which raised up to 38.4 % of the cladode, whit a severity catalogued as "Very high", and a minimum loss of 0.77 % at "Very low" severity. In general, 46.1 % of the cladodes lost between 0.77 and 9 % of the photosynthetic area, and 42.6 % of them lost between 9 and 19 %. Heart-shaped cladodes reduced an average photosynthetic area of 8.6 % (37.6 cm²), with an average damage width of 8 cm and 5.6 cm of invagination depth.

Loss of buds in cladodes of Opuntia ficus-indica cv. Villanueva

The asymptomatic cladodes presented between 15 and 29 apical buds, while in heart-shaped cladodes the number of buds decreases to values between 4 and 17. Also, 74.4 % of the cladodes lost 51 to 81% of their buds, and only 25.6 % lost between 15 and 50% of apical buds. With no doubt, this loss of buds affects negatively the production of new cladodes, which in turn will originate a lower number of viable apical buds.

Regarding the severity of other diseases, such as clorotic spots, values between 51 and 79% have been registered (Alonso-Barrera *et al.*, 2015). Results obtained in this study showed incidences between 12 and 30%, and degrees of severity below 38%, causing an average photosynthetic area loss of 8.6%, which are lower than those reported for other diseases, such as thickening and black spot (Quezada-Salinas *et al.*, 2006; Hernández-Pérez *et al.*, 2009).

CONCLUSIONS

Three main symptoms characteristic of heart-shaped cladodes were found in the cactus pear plantations studied: "heart", "bean" and "saw", with the first one being the predominant at the plant level and among cladodes.

The presence of symptoms in both Pinos and Villanueva, Zacatecas, showed a broad regional, varietal, seasonal and within-plots prevalence.

The incidence and severity of heart-shape cladode symptoms can reduce the productivity of cactus and cause important monetary losses to producers of either nopalitos or fruit.

As a recommendation, it is necessary to carry out studies that allow the identification of the causal agent of this malformation to implement a management strategy including sanitary pruning and strict selection of propagation material, among other practices, to reduce its epidemic expression in other zones and the resulting economic impact.

REFERENCES

- Alonso-Barrera, B., Mora A., G., Valdovinos P., G., Ochoa M., D. L., Rodríguez L., E., Tlapal B., B., De la Torre A., R. 2015. Asociación de un potexvirus como agente causal de manchas cloróticas en *Opuntia ficus-indica*. *Revista Mexicana de Fitopatología* 33: 75-86.
- De Jesús G., A. B., Aragón G., A., López O., J. F., Rivera, A., López M., V. 2016. Entomofauna asociada al nopal verdura (*Opuntia ficus-indica* Miller) en San Andrés Cholula, Puebla, México. *Southwestern Entomologist* 41(1): 259-266. http://dx.doi.org/10.3958/059.041.0123.

- Dewir, Y. H., El-Mahrouk, M., Omar, A. F. 2015. Molecular characterization of 16SrII phytoplams group and associated down-regulation of ABA and IAA in *Opuntia caracasana*. *Phytopathogenic Mollicutes* 5(2):100-106.
- Dewir, Y. H. 2016. Cacti and succulent plant species as phytoplasma hosts: A review. *Phytopathogenic Mollicutes* 6 (1):1-9
- Fucikovsky, Z. L., Yañez M, M. J. 2006. Presence of phytoplasma in edible cactus, insects, snails, and weeds. *In*: Proceedings of the 11th International Conference on Plant Pathogenic Bacteria. Edinburgh, Scotland. United Kingdom. Abstract A p. 29.
- Fucikovsky Z., Yañez-Morales, M. J. Alanís-Martínez, I., González-Pérez, E. 2011. New hosts of 16Srl phytoplasma group associated with edible *Opuntia ficus-indica* crop and its pests in Mexico. *African Journal of Microbiology Research* 5(5):910-918. DOI:10.5897/AJMR10.846.
- Granata, G. 1995. Biotic and abiotic diseases. *In: Agroecology, Cultivation and Uses of Cactus Pear.* Barbera, G., Inglese, P., Pimienta-Barrios, E. (ed.) FAO. Plant Production and Protection132:109-119. ISBN:92-5-103735-3.
- Granata, G., Sidoti, A. 2002. Survey of diseases discovered on *Opuntia ficus-indica* in producer countries. *Acta Horticulturae* 581:231-237. https://doi.org/10.17660/ActaHortic.2002.581.24
- Granata, G., Paltrinieri, S., Botti, S., Bertaccini, A., 2006. Aetiology of *Opuntia ficus-indica* malformations and stunting disease. *Annals of Applied Biology* 149:317-325. doi:10.1111/j.1744-7348.2006.00097.x
- Hernández-Pérez, R., Noa-Carrazana, J. C., Gaspar, R., Mata, P., Flores-Estevez, 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.
- Hernández-Sánchez, E, Mora-Aguilera, G., Tlalpal-Bolaños, B., Rodríguez-Leyva, E., Alvarado-Rosales, D. 2014. Efecto de intensidad inicial de enfermedad en la caracterización temporal y espacial de la mancha negra del nopal (*Opuntia ficusindica*). Revista Mexicana de Fitopatología 32:132-146.
- López-Gutiérrez, D. M., Reyes-Agüero, J. A., Muñoz, A., Robles, J., y Cuevas, E. 2015. Comparación morfológica entre poblaciones silvestres y manejadas de *Opuntia atropes* (Cactaceae) en Michoacán, México. *Revista Mexicana de Biodiversidad* 86:1072-1077. http://dx.doi.org/10.1016/j.rmb.2015.08.006.
- Mendoza-Orozco, M. E., Hernández-Ríos, I., Morales-Flores, F. J., Mena-Covarrubias, J., Méndez-Gallegos, S. de J. 2014. Estudio de la sintomatología del 'acorazonamiento de cladodios' de *Opuntia* spp. *In*: Blanco-Macías, F., Vázquez-Alvarado, R.E., Valdez-Cepeda, R.D., and Santos-Haliscak, J.A. (Eds.). Memorias XIII Simposium-Taller Nacional y VI Internacional Producción y Aprovechamiento del Nopal y Maguey. Escobedo, Nuevo León. pp: 229-235.
- Osorio A., F., Soto E., A., Méndez G., S. de J. 1997. Manejo de huertas de nopal (*Opuntia* spp.) para el control del "engrosamiento de cladodios" y de la producción. *Agrociencia* 31:67-72.
- Pimienta B., E. 1990. El Nopal Tunero. 1ª ed. Universidad de Guadalajara. Guadalajara, Jalisco, México. 246 p. ISBN:968-895-140-4.

- Quezada-Salinas A., Sandoval-Islas, S., Alvarado-Rosales, D., Cárdenas-Soriano, E. 2006. Etiología de la mancha negra del nopal (*Opuntia ficus-indica* Mill) en Tlalnepantla, Morelos, México. *Agrociencia* 40:641-653.
- Quezada S., A., Sandoval I., J. S., Alvarado R. D., Moreno V. M. 2013. Histopatología y patogénesis de *Pseudocercospora opuntiae* en nopal. *Revista Mexicana de Micología* 38:9-18.
- SIAP (Agricultural and Fishery Information System). 2017. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México. http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-estado/. (Accessed on February 28, 2017).
- Suaste D., A., Rojas M., R. I., Zavaleta M., E., Pérez B., D. 2012. Detección molecular de fitoplasmas en nopal tunero (*Opuntia ficus-indica*) con síntomas de engrosamiento del cladodio. *Revista Mexicana de Fitopatología* 30(1): 81-85.
- Swart, W. J., Swart, R. V. 2003. An overview of research on disease of cactus pear in South Africa. *Journal of the Professional Association for Cactus Development* 5: 115-120.
- Zheng-Nan, L., Lei, Z., Ping, L., Yao-Bo, B., Xiao-Gang, Y., Yun-Feng, W. 2012. Detection and molecular characterization of cactus witches'-broom disease associated with a group 16SrII phytoplasma in northern areas of China. *Tropical Plant Pathology* 37 (3):210-214.