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Alternaria alternata and *Phoma* spp., associated with Necrotic Spot Syndrome in Giant Cardon in Baja California Sur, Mexico

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Abstract. Cardon cacti (*Pachycereus pringlei*) are an important forest resource in Baja California Sur (B.C.S.), playing a vital role in Mexico's arid zones and carrying ecological, cultural, floristic, and social significance. Over the past two decades, fungal diseases, presumably linked to a necrotic spot syndrome (NSS), have been recorded in the region, causing significant damage to wild populations of this iconic columnar cactus. The causative agents were identified through morphological and molecular characterization, and disease pathogenicity was assessed by measuring severity, incidence, and spatiotemporal recurrence. We identify *Alternaria alternata* and *Phoma* sp. associated with necrotic spots on wild cardons, which cause dark brown spots, pustules with a chlorotic halo, small chancres, and concentric circle lesions. Disease intensity of 52.3% can be considered high, with an incidence of 65.3% and a severity of 39.5%. Severity estimates from two independent methods (diagrammatic and lesion range-based) were similar, indicating that both methods can be used to estimate severity. To verify pathogenicity, experimental inoculations were performed to satisfy Koch's postulates, and the resulting symptoms were similar to those observed in the field. This study confirms the presence and spread of fungal diseases in cardon cacti and identifies *Alternaria alternata* and *Phoma* sp. as causal agents of the disease NSS. This study contributes to the development of conceptual strategies for preventive actions against fungal diseases in giant cardon cacti in arid zones of B.C.S., Mexico, and worldwide.

Keywords: *columnar cacti, fungal syndrome, arid zones, phytosanity*

Introduction

The giant columnar cacti of the genus Pachycereus are found throughout Mexico and are among the country's most representative endemic plants. This genus includes eight species, including *Pachycereus pringlei* (S. Watson) Britton & Rose, the most widely distributed in Northwestern Mexico, primarily in the Sonoran Desert and along the Baja California Peninsula (Arias and Terrazas, 2009). Wild cardon cacti typically occupy ecological niches in areas of great aesthetic, spiritual, and cultural value, and their ecological importance helps maintain the flow of matter and energy within the ecosystem (Bravo-Hollis and Sánchez-Mejorada, 1991; Anderson, 2001). Field evidence of fungal damage in columnar cacti is limited, as most pathological issues have been recorded in other commercially important cacti, such as prickly pear (genus Opuntia), and are principally confined to Puebla and Oaxaca (Chavarria-Cervera *et al*., 2024). However, other biotic factors, such as insect damage, particularly by Coleoptera larvae, have increased secondary infections by facultative fungi that may kill entire plants (Castellanos *et al*., 2010; Ramírez-Delgadillo *et al*., 2011; Maya *et al*., 2011; Bravo-Aviléz *et al*., 2019). In the peninsular region, the occurrence and spread of fungal diseases have historically been linked to flat-top decay syndrome (Bashan *et al*., 1995), but no causal agent has been identified. Almost two decades later, the fungus *Bionectria* spp. was associated with apical stem rot diseases in *P. pringlei* in the same area (Holguín-Peña *et al*., 2012). However, brownish-gray necrotic spots and concentric dark spots continue to be observed in cardon cacti in several ecosystems in the municipalities of La Paz and surrounding areas, where previous morphological characterizations have presumed the nature of the fungal infection (Medina-Hernández *et al*., 2017; Holguín-Peña *et al*., 2018; CONAFOR, 2024; Medina-Hernández, 2025).

The objective of this study was to identify the causal agents of a necrotic spot disease in cardon cacti (*P. pringlei*) in an experimental area that represents the cactus floristic niches of Baja California Sur and northwest Mexico. It also aimed to determine disease parameters: i) recognition of symptom patterns, ii) estimation of incidence, iii) estimation of severity using diagrammatic scales, and iv) estimation of severity based on the type, number, and size of lesions. This study contributes to understanding the phytosanitary status and confirms that fungal agents cause necrotic spot disease, providing a foundation for future efforts to conserve this iconic biological and floristic resource, an icon of Mexico and a heritage of the world's deserts.

Materials and Methods

Study area

The study area was “Dra. Laura Arriaga Cabrera” Biological Station of Centro de Investigaciones Biológicas del Noroeste (CIBNOR), located in El Comitán, La Paz, Baja California Sur (24°08′ 00.8″ N, 110° 25′ 54.3″ W) (Fig. 1). The area has minimal human activity and vegetation typical of desert plant communities on the Baja California peninsula and in northwestern Mexico. The biological station covered 3,360 m², where necrotic fungal-like diseases have been observed historically. To represent the phenomenon, 10 experimental units (quadrants), each measuring 20 × 20 m, were randomly selected.

Recognition of disease patterns

A preliminary assessment was conducted to observe symptoms associated with necrosis syndrome. In each quadrant, symptom types and distribution patterns were estimated from descriptions of the disease. The damage observed mainly consisted of concentric necrotic spots ranging from 0.5 to 5 cm in diameter on the main or secondary branches, consistent with reports for this disease (Kin *et al*., 2000; Zimmermann and Granata, 2002; Deb *et al*., 2020; Chavarria-Cervera *et al*., 2024). The visual inspection was based on lesion size (0.5 to 7 cm) observed in *Opuntia* cacti (Horst, 2001; Swart and Swart, 2003) and divided into four categories: pustules, chancres, anthracnose, and large lesions (Flores-Flores *et al*., 2013; Hernandez-Sánchez *et al*., 2014; Oliveira *et al*., 2018). Additionally, previously described necrotic symptoms in the area (Holguín-Peña *et al*., 2018) were considered.

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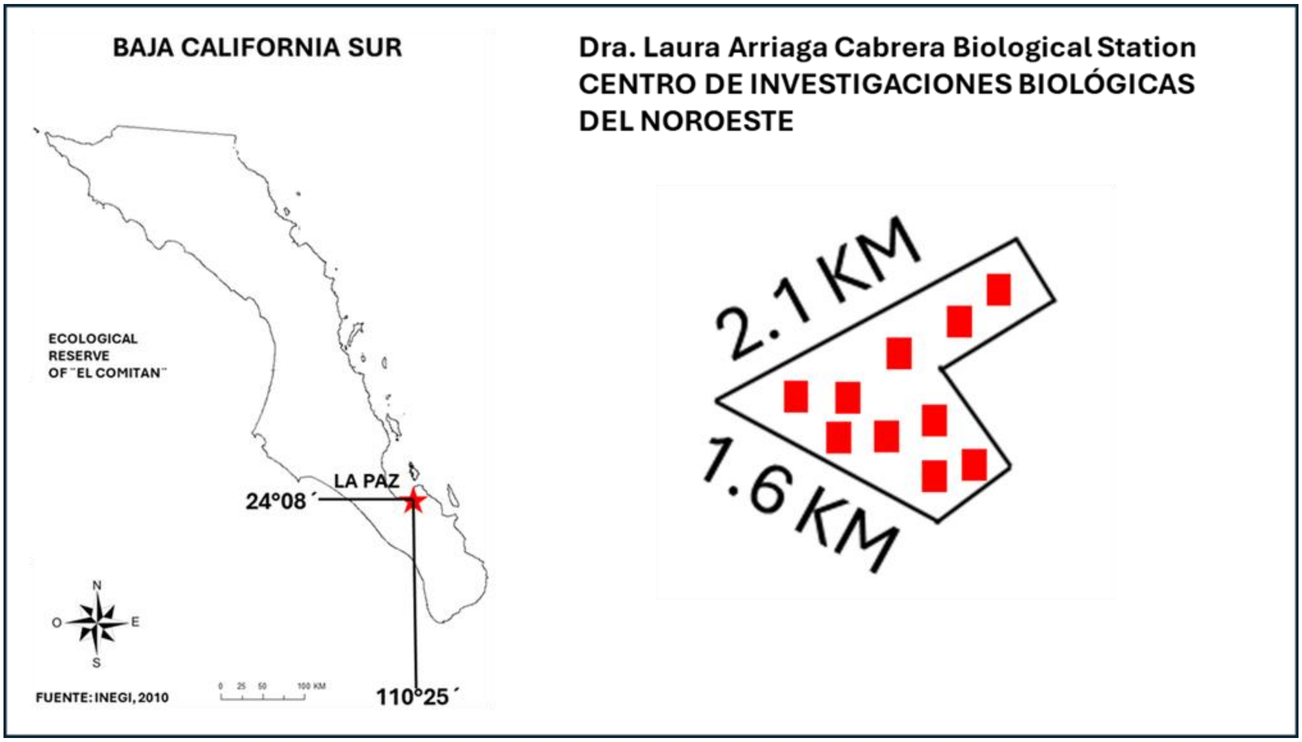

Figure 1. Schematic map indicating the geographical points of the ecological reserve "Dra. Laura Arriaga Cabrera" Biological Station of Centro de Investigaciones Biológicas del Noroeste (CIBNOR), located in El Comitán, La Paz, Baja California Sur (24°08′ 00.8″ N, 110° 25′ 54.3″ W). The irregular hexagon represents the 2.1 × 1.6 km of the reserve. Red squares represent the experimental quadrants (20 m × 20 m).

Fungal collection

Based on recorded symptoms, 0.5 cm explant tissue was collected from a representative stem of cardon cacti in each of the ten quadrants. The plant collection was performed using sterile tools, with care to minimize damage to the specimen. The samples were placed in paper bags, transported to the phytopathology laboratory of CIBNOR for fungal isolation and molecular analyses, then frozen in liquid nitrogen and stored at -80 °C. After morphological characterization, the samples were cultured on agar-water medium to obtain pure cultures for subsequent molecular identification and pathogenicity testing. To avoid contamination and saprophytic species, the tissues were disinfected for 2 minutes, 90, 60, and 30 seconds with 10% hypochlorite; after rinsing with sterile water, they were seeded in Petri dishes with agar-water culture at 3%. When growth was observed in the isolates, the fungi were reseeded onto PDA (potato dextrose agar) medium containing streptomycin sulfate and oxytetracycline, and the cultures were incubated in a growth chamber at 28 °C for 4 to 8 days. For morphological identification, colonies at 6 to 8 days of development were sampled, stained with cotton blue in lactophenol, and observed with a Carl Zeiss® brightfield compound microscope using 10x, 40x, and 100x objectives (Chavarria-Cervera *et al*., 2024).

Molecular identification

A portion of the mycelium grown on PDA was frozen in liquid nitrogen, ground in a mortar, and subjected to the modified CTAB total DNA extraction protocol. Extracted DNA was quantified using a Nanodrop One (Thermo Fisher Scientific), and integrity was assessed on a 1% agarose gel (Sigma-

Aldrich, Merck) stained with SYBR Safe, using a 1 kb Plus DNA Ladder (Invitrogen, TFS) as the molecular weight marker. Fungal molecular identification was performed using the internal transcribed spacer (ITS) region, with oligonucleotides ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA - 3′), ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'), ITS3 (5'- GCATCGATGAAGAACGCAGC-3'), ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), and ITS4B (5'-CAGGAGACTTGTACACGGTCCAG-3'), with PCR conditions according to White *et al*. (1990). Amplicons were visualized by electrophoresis on a 1% agarose gel, and the purified products were then sequenced at GENEWIZ (Azenta Life Sciences). Sequences obtained from the GenBank database were aligned using Multiple Sequence Alignment (MSA) by Clustal Omega, and identities were determined using the BLAST tool (Morgulis *et al*., 2008) at the National Center for Biotechnology Information (NCBI). To compare the relationship with other fungi isolated in the region or associated with cardon disease, a phylogenetic tree was constructed using maximum-likelihood and Bayesian inference methods with the Phylogeny.fr program (Zhang *et al*., 2023).

Incidence determination

The incidence of the disease is the proportion of plants with injury or damage from brown spot disease, expressed as a percentage. These metrics were calculated using the formula: Inc (%) = (DP × 100)/T, where DP = diseased plants and T = total plants evaluated. A cardon cactus was considered to have the disease if any of the symptoms described for *Alternaria* spp. and/or *Phoma* spp. were present (pustules, cankers, concentric medium spots, elongated black spots). The final incidence for the population was calculated as IncT = (IncQ + IncS)/2, where IncQ = incidence by quadrant and IncS = incidence by symptom. Given the limited information on cardon cactus, symptom recognition was based primarily on reports of fungal diseases in cactus pear.

Severity based on diagrams and lesion classification

Severity is the percentage of tissue affected in a diseased plant. In this study, severity was estimated using two complementary methods: diagrammatic values (SevD) and severity ranges (SevR). Diagrammatic methods rely on visual inspection of damage and assign five severity levels (Fig. 2), with 0 indicating healthy plants and 5 indicating severely affected plants. Severity ranges are based on the number and size of typified lesions (pustules, chancres, anthracnose, large necrotic lesions), which can represent 0, 15, 30, 50, 80, and 100% of the disease. This parameter was calculated using the formula SevR = $\sum$d/n, where "d" is the number of infected plants in each range and "n" is the number of values in each level (0-16). In Table 1, we set out the criteria for lesion size and symptom count within each range. The number of lesions in each range was from 0 to 16. The "d" value for each lesion size (columns) was based on lesion sizes reported in spot lesion diseases (≤ 1 cm) (Anderson *et al*., 2001; Da Silva *et al*., 2021) and in canker tree diseases (2-7 cm) (Pinkerton *et al*., 1993). Population severity (SevP) is the sum of the total severity observed in the field and was calculated as SevP = $\sum$Sevq/T, where Sevq is the severity in each quadrant, and T is the total number of quadrants. Disease intensity (ID) determines the maximum level of damage in the population, accounting for cumulative severity and incidence while considering damage by quadrant and for each observed symptom per specimen. The additive effect of severity and incidence more accurately indicates the disease's impact and progression over time.

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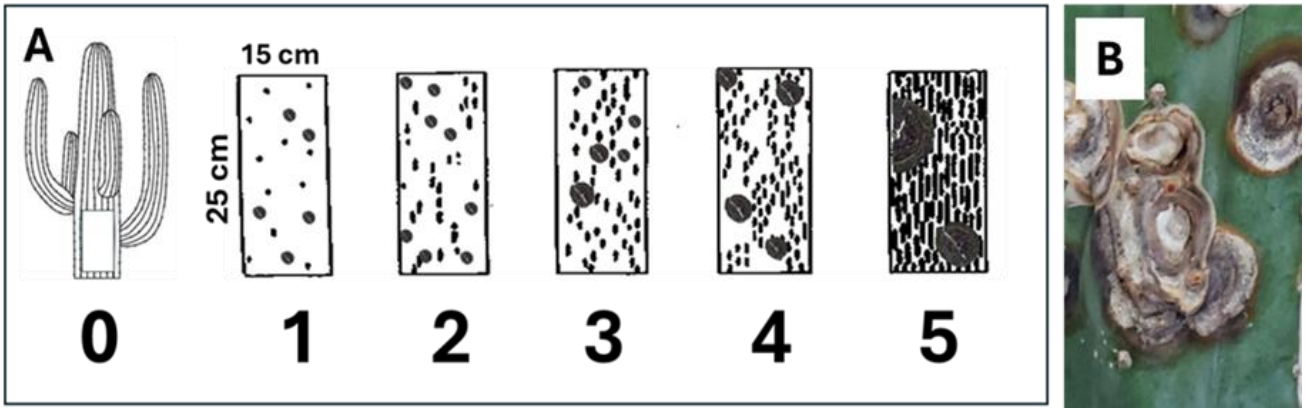

Figure 2. Criteria for estimating the severity in cardon cactus *Pachycereus pringlei.* **A**. Diagrammatic scale based on the number and size of lesions in an area of 15×25 cm in the first third of the main stem, where 0 represents plants with no evidence of fungus damage, and 5 = plants with severe symptoms and difficult to recover. **B**. The symptoms of concentric spots are quantified as lesions less than 1 cm (Oliva *et al*., 2013).

Pathogenicity test

To determine the nature of the disease, pathogenicity tests and Koch's postulates were conducted. The inoculum was the previously isolated, purified, and morphologically characterized fungus. *Phoma* sp. and *A. alternata* were inoculated into healthy cardon plants. Each inoculation test used six 10-15 cm, two-month-old plants in 2-liter pots, with three replicates. Inoculation was performed by making vertical knife wounds and inserting a fragment of a pure colony of the respective fungus. The wound was covered with cotton moistened with sterile water. All procedures were performed under sterile conditions. After inoculation, each cactus was placed in a growth chamber at 25 °C and 50% humidity, with a 12/12 h light/dark cycle. Parameters were measured, and symptoms were analyzed over two months, with plants monitored daily and photographed every 10 days.

Results

Disease recognition

The observed symptoms were classified and associated with necrotic spot disease. All lesions measured from 0.3 cm to 7 cm, as indicated in Table 1. The most common symptoms associated with the disease were necrotic dots with a dry appearance, located beneath the tissue and extending up to 3.5 mm toward the cuticle. Figures 3A and 3E show symptoms associated with necrotic disease in mature cacti, with the most characteristic lesions being concentric ring patterns and circular pustules ranging in size from 0.5 to 1.5 cm and in color from beige to brown. These symptoms clearly differentiate cankers, anthracnose, scab, and elongated lesions that are larger than 1.5 cm and up to 9 cm in length. Some lesions showed chlorotic halos around the affected area, while others presented as concentric pustules (Fig. 3). This condition implies that the disease may involve lesions of varying sizes. The highest concentration and clustering of symptoms were observed on the main stems at an average height of 1.7 m from the ground. In some cases, a black center could be observed, and eventually, a fungal sign appeared around the pustules. However, in most lesions measuring 2 to 4 cm, a flat morphology with dry rot and a brown-to-black appearance was observed. Although less frequent, anthracnose-type symptoms with dry, yellowish, and dark lesions in the thorns were observed in older cardons.

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Table 1. Severity ranges and categories, based on lesion size and number, to estimate damage from necrotic disease in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico.

SevR [1]	**Range and category** [2] lesion size (*d*) / number of lesions				
(%)	≤ 1cm	1-3 cm	4 cm (±1)	6 cm (±1)	≥7 cm
0	0	0	0	0	0
15	1	1	1	1	1
30	4	3	2	1	1
50	8	6	4	2	2
80	12	9	6	3	2
100	16	12	8	4	3

[1] SevR = Severity ranges estimated in percentages.
[2] Ranges by lesion size (≤ 1 cm to 7 cm) and by number of lesions (0 to 16). The d value for each lesion size (columns) was based on spot lesions ≤ 1 cm (Oliva *et al*., 2013) and on canker-like lesions of 2-7 cm on trees (Pinkerton *et al*., 1993).

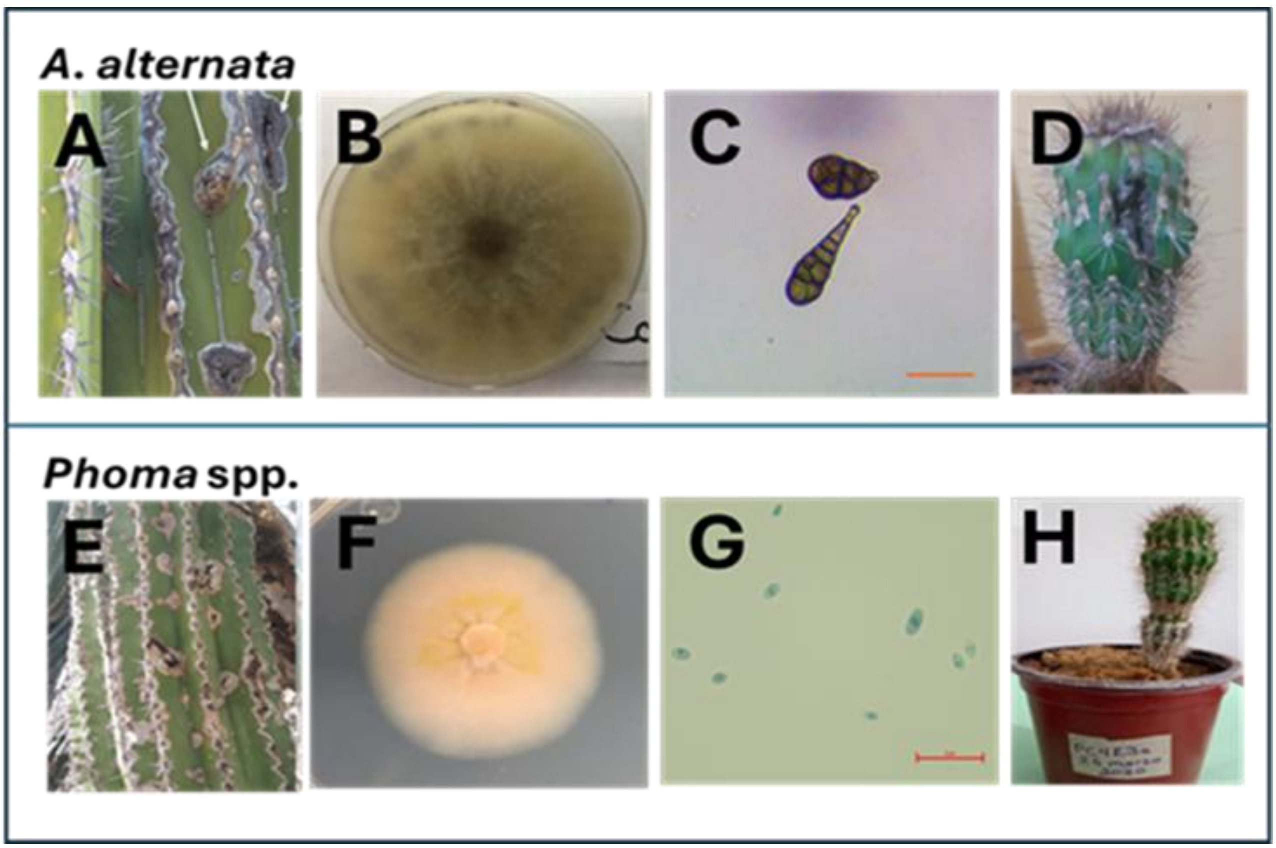

Figure 3. Necrotic disease caused by a mixed fungal infection in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico. **A)***. Alternaria* spp. exhibiting necrotic pustules and concentric dots. **B)** *Alternaria* in PDA cultures with radial growth of cottony mycelium. **C)** Conidia at 40x with the presence of a longitudinal septum, elliptical-ovoid in shape, measuring 20-50 × 10-18 µm in size. **D)** Re-infecting protocol in young cardons, exhibiting symptoms of necrotic spots with a concentric pattern. **E)** *Phoma* spp., in natural field infection. **F)** *Phoma* in PDA medium, forming dense mycelial colonies with a woolly appearance. **G)** Presence of unicellular hyaline conidia (5-8 × 1-3 µm in size), containing one or two oil droplets inside. **H)** Re-infection with *Phoma* inoculum in young cacti, exhibiting symptoms of dry-appearing necrotic spots.

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Morphological characterization and molecular identification

Based on observed symptoms, morphological characterization, and molecular tests, the causative agents of the disease were *Alternaria cartami*, *A. alternata*, and *Phoma* spp. Morphological studies of fungi isolated from diseased cardon cacti revealed at least two types of filamentous fungi, which were purified and prepared for molecular testing. In the coloration test of mycelium growth for *Alternaria* spp., the mycelium was earthy brown and had a cottony appearance (Fig. 3B). In *Phoma* spp., we observed olive gray to brown coloration in 5-day-old PDA cultures (Fig. 3F). Of the 24 collected fungi, we observed two distinct groups in relation to the characteristic symptoms; some exhibited necrotic lesions with concentric patterns, and others showed necrotic dots with a surrounding halo. However, most isolates exhibited circular growth with a flat elevation and a cottony texture. Colony growth time also varied; some covered the Petri dish surface within the first five days, while others did so after 10 days. In *Alternaria*, radial growth with a slightly brown color, septate mycelium, and multicellular, brown conidia arranged in long chains was observed (Fig. 3B). In *Phoma*-like isolates, hyaline, rose-pale mycelia were observed, with 2-4 µm unicellular, ovoid or ellipsoidal, hyaline conidia (Fig. 3G). DNA comparison of the analyzed samples identified the species as Alternaria alternata (Fr.) and *Phoma* sp. The taxonomic identification of fungal isolates was confirmed to be 98–100% identical to GenBank sequences (Fig. 4A). The results revealed that both fungi are associated with necrotic spot syndrome (NSS) in cardon cactus and are involved in necrotic physiological disorders. The DNA sequences were deposited in the GenBank database, two for *A. alternata* (MZ572939) and one for *Phoma* sp. (MZ572934). The identification was based on 98% nucleotide similarity to the reference isolates for *Phoma* sp. and *A. alternata* (Fig. 4A). Furthermore, comparative analyses of the BCS isolates, using maximum-likelihood and Bayesian inference, established that their closest relatives are other species of *Alternaria* and *Phoma* and that they are phylogenetically distinct from other necrotrophic fungi isolated in the region, such as *Bionectria* spp. (Fig. 4B).

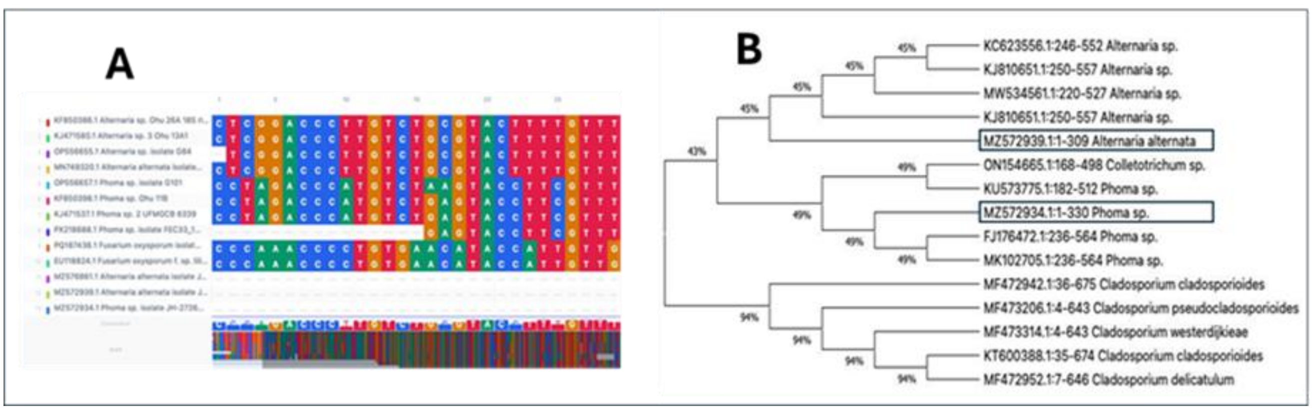

Figure 4. Comparative analyses of *Alternaria* (MZ572939) and *Phoma* (572934) isolates in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico and associated with necrotic spot syndrome (NSS) in cardon cacti. **A**) Multiple sequence alignment (MSA) by Clustal Omega; the letters and colors represent the nucleotide identity of the BCS isolate with respect to *Alternaria* and *Phoma* isolates from GenBank. **B)**. Phylogenetic tree of evolutionary relationships among fungal species based on the [Maximum Likelihood / Bayesian Inference] method, using sequences from the internal transcribed spacer (ITS) region of ribosomal DNA. Reference sequences were obtained from the GenBank database.

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Incidence and severity

The total incidence (65.36%) was derived from the symptom-type incidence of 48.43% (Table 2) and the quadrant incidence of 82.3% (average across 10 quadrants) (Table 3). Across all quadrants, incidence averaged 60%, ranging from 57.5% to 100%. On average, 64.17% of lesions were smaller than 1.5 cm and were classified as pustules and cankers. Across symptom groups, the incidence was 48.43%, with the highest at 57.75% for necrotic pustule-type lesions and 44.15% for anthracnose-type lesions. The lowest incidence, by symptom type, was 21.22% for elongated, coalescing necrotic lesions. Total severity was 39.50%, calculated as the average of the diagrammatic severity and that assigned based on the size, number, and type of injury. The severity, as measured by the diagrammatic scales (SevD), was 40%. The severity by range, size, and number (SevR) was 39.01%, with the highest values in severity due to pustules (46.2%) and cankers (56.54%) (Table 4). The total severity estimated differed by 1%. Both criteria were related to damage severity, whether in individual measurements or in quadrant analyses estimating population-level damage. The severity index, estimated by ranges, was highest (51.37%) for pustule and canker lesions. These symptoms were the most observable and recognizable in the field and among the most strongly associated with necrotic spot disease. Other common symptom types observed in the population included dots, dry cankers, ring spots, cracked and concentric circle lesions, necrotic spots, and anthracnose. As confirmed by pathogenicity tests, these lesions were caused by *A. cartami*, *A. alternata*, and *Phoma* spp., as well as by other filamentous fungi not included in this study. Tables 3 and 4 summarize the data collected in this study for the pathological analysis of severity. The number of specimens, the presence or absence of affections in each, and the weighting of severity by affection are reported for each quadrant.

Table 2. Disease severity levels by quadrant in wild populations of cardon cacti in La Paz, Baja California Sur, Mexico.

	Incidence[1]		**Diagrammatic severity (SevD)[2]**	
Quadrants	**Diseased/total**	**%**	**Scale (1-5)**	**%**
1	2.7/3	90	1.4	29.4
2	5.7/6	95.8	1.6	33.8
3	5.7/3	95.8	2.2	45.0
4	6/8	75	1.6	32.4
5	4/4	100	2.3	46.6
6	5/7	71.4	1.4	28.8
7	4.5/6	75	1.8	36.0
8	3/4	75	2.9	58.0
9	1.7/2	85	3.1	63.0
10	1.2/2	60	1.3	27.4
Total	3.9/4.5	82.3	2	40

[1] The incidence is the number of diseased plants in each quadrant.

[2] Diagrammatic severity was calculated with the formula: SevD (%) = scale (100)/n, where scale = damage value in each quadrant, n = the maximum value of the scale (5). 0 = plants without visible symptoms

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Table 3. Incidence of brown spot-type symptoms associated with necrotic cactus diseases in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico.

S[1]	**Diseased cacti by quadrant (PS/T)**[1]										**Inc %**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Pustules	3/3	6/6	6/6	8/8	4/4	7/7	6/6	4/4	2/2	2/2	57.7
Cankers	3/3	6/6	6/6	8/8	4/4	7/7	6/6	4/4	2/2	2/2	70.6
Anthracnose	3/3	6/6	5/6	4/8	4/4	6/7	5/6	4/4	2/2	1/2	44.1
Necrotic large lesions	2/3	5/6	6/6	4/8	4/4	0/7	1/6	0/4	1/2	0/2	21.2
Total (T)	2.7/3	5.7/6	5.7/6	6/8	4/4	5/7	4.5/6	3/4	1.7/2	1.2/2	48.4

[1] S = Symptoms associated with necrotic disease.
[2] T = Total percentage of diseased cacti across the 10 quadrants, with an infected plant defined as exhibiting at least two symptoms of necrotic disease (Chavarria-Cervera *et al*., 2024).

Table 4. Diversity of symptoms and severity of the fungal agents causing the cactus necrotic spotting syndrome (CNSS) in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico.

Symptoms [1]		**Severity** [2]		**Fungal causal agents**
Category	**Type**	**Range**	**(%)**	
Pustules	Dt, Sp	2.31	46.2	*Alternaria carthami*
Chancres	DCh, Dt, Rs, CCL, Ns, An	2.82	56.54	*Phoma* spp. *Alternaria alternata*
Concentric medium spots	CCL, An, Bs, ISh, Ns	1.76	36.32	*Alternaria* spp. *A. alternata* *Phoma* spp.
Elongated black spots	LDL, Ish, DP	0.85	17.01	*Alternaria* spp.
Total		1.93	39.01	*Alternaria* spp. + *Phoma* spp.

[1] Category is the symptoms reported as associated with the brown dot cactus disease. Type symptoms include dots (Dt), speckles (Sp), dry chancres (DCh), ring spots (Rs), cracked lesions (CrL), concentric circle lesions (CCL), anthracnose (An), brown spots (Bs), irregular-shaped lesions (ISh), necrotic spots (Ns), large dark-shaped lesions (LDL), and dark patches (DP).
[2] Severity of the necrotic disease based on ranges (0 to 5) and percentages, where 100% = plants severely affected and difficult to recover.

Pathogenicity test

Pathogenicity tests were positive using inoculum of *Phoma* sp. and *A. alternata* isolated from diseased plants. The sexual structures (conidia) observed in the inoculated plants matched those in the field isolates. Figure 3 (D, H) shows the pathogenic effects of the *Alternaria* isolate, associated with necrotic spot syndrome or cankers, and it is molecularly identified as *Phoma* spp. The expected symptoms of both fungi were visible after 10 days, including circular brown spots, discoloration, loss of cellular structure, dermal thinning, and subsequent darkening of some areas of the stem. By 10 days, the infected cardon had lost its structure and turgidity, and by 60 days, the aerial part appeared as a single gray crust with white dots, primarily in the area surrounding the ribs. No symptoms were observed in the non-inoculated plants, even after the bioassay was completed.

Discussion

Fungal agents *A. alternata* and *Phoma* spp. were associated with the necrotic spotting syndrome (NSS) in wild cardon cactus (*P. pringlei*) in a nature reserve in La Paz, B.C.S., Mexico. Both filamentous species are well documented to cause diseases in cultivated plants, with typical symptoms including circular dark brown spots, dry pustules, small chancres, and concentric circles (Pérez-Vera *et al*., 2005). In older cactus pear (nopal) plants (*Opuntia ficus-indica* Mill.), these symptoms can coalesce to form elongated necrotic lesions, which are often mistaken for other necrotic disorders (Choi *et al*., 2010; Chavarria-Cervera *et al*., 2024). The Biological Reserve "Dra. Laura Arriaga Cabrera" is an appropriate study model because it contains wild populations of cardon cacti with symptoms of NSS disease. This study highlights the pathological condition of the columnar cactus, which has been affected by fungal disease for at least two decades (Bashan *et al*., 1995), but it does not identify the causal agents or define the role of fungi in the disease's epidemiology. Another critical point is the historical absence of methodologies for estimating damage, the reliance of severity assessments on subjective judgments to assign values, and the inherent difficulty of measuring a physiological response when it lacks a normalized trend and when some epidemiological components are non-parametric (Lannou, 2012). In the present study, we complement two methods for estimating severity: assigning intensity-based ranges and visually estimating damage, and we also create diagrammatic scales, which are useful because they make field data easier to interpret (Bock *et al*., 2022). According to our studies, the estimated severity from diagrams and ranges differed by 1%, indicating that both methods can be used to estimate disease intensity. The severity of 39.5% in this study was high, corresponding to a predictive score of 1.9 on a 5-point scale. Moreover, evidence shows that these symptoms have persisted in the region for at least five years since the first recorded symptoms of the disease (Holguin-Peña *et al*., 2021).

Black spot symptoms caused by *Alternaria* diseases have been observed on prickly pear nopal (*Opuntia ficus-indica* Mill.) in other arid regions of Mexico, specifically the states of Morelos and Colima (Flores-Flores *et al*., 2013; Chavarria-Cervera *et al*., 2024). Our findings align with the prevalence of *Alternaria* spp. and *Phoma* spp., which are associated with the NSS disease. Other fungi, such as *Corynespora cassicola*, *Neoscytalidium dimidiatum*, *Colletotrichum gleosporioides*, *Curvularia lunata*, and *Fusarium lunata*, have been found associated with related necrotic-type disorders (Bravo-Avilez *et al*., 2020; Chavarria-Cervera *et al*., 2024). *Alternaria* is among the most widespread fungi associated with diseases in horticultural crops and forest environments. As observed in this study, they cause a diverse range of symptoms, including concentric black spots, dry cankers, and, less frequently, dieback and black rot (Canihos *et al*., 1999; Troncoso-Rojas and Tiznado-Hernández, 2014; Raya-Pérez *et al*., 2018). In ornamental cactus (*Gymnocalycium mihanovichii*), symptoms can include faint concentric circles and dark brown spots, particularly on the areolas of the stems (Pérez-Vera *et al*., 2005; Choi *et al*., 2010). In other cactus species, some lesions can coalesce over time, forming larger necrotic areas that can eventually perforate the stems (Evans and Macri, 2008; Conforto *et al*., 2019; Infante *et al*., 2021). In pathogenicity tests using *Alternaria* inoculum on 5-week-old cacti, we observed circular brown-to-black spots similar to those observed in wild populations in the biological reserve. We also observed rapid progression of the infection, with evident symptoms within the first week. This response aligns with reports that this genus is highly resilient, capable of surviving in extreme conditions, and capable of infecting host tissues within 3 to 72 hours (Dehpour *et al*., 2007).

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The genus *Phoma* has long been taxonomically controversial, with over 3,000 taxa described, including 110 pathogenic species that occupy various ecological niches (Chen *et al*., 2015). In the study area, we observed that *Phoma* caused canker symptoms and necrotic pustules, although it has also been reported to cause leaf blight, root rot, and wilting in other cultivated plants (Deb *et al*., 2020). *Phoma* spp. have been reported in some cacti, such as saguaro and nopal, both as endophytic fungi and as pathogens, and have been reported on *Cereus peruvianus*, which exhibits similar pustule symptoms (Suryanarayanan *et al*., 2005; Perales-Segovia *et al*., 2018). Frequently, different species of *Phoma* have been associated with other tropical species, such as *Phoma costarricensis*, which causes burning disease in coffee plants and necrotic lesions in conifer seedlings, including *Pinus sylvestris*, *Pinus sibirica*, and *Picea obovata*, resulting in premature leaf abscission and dark-brown discoloration with necrosis in lignified areas and eventually leading to descending death (Sheller *et al*., 2020).

Disease intensity in this study can be considered high, based on historical severity, incidence, and recurrence observed in the region over the years (Holguin-Peña *et al*., 2018). Elucidating symptoms and severity in the field is difficult, particularly when multiple causative agents may be involved in the same disorder or syndrome, such as NSS disease (Lima *et al*., 2011; Djebali, 2013; Del Ponte *et al*., 2017; Buja *et al*., 2021; Kaweesinsakul and Nuchitprasitchai, 2021). Severity is a non-parametric response that cannot be modeled with a normal distribution; therefore, it should be measured by assigning discrete values to qualitative data (Chiang and Bock, 2022; Habib *et al*., 2022). These criteria for estimating damage in cacti have not been widely applied to cardon cacti because of their limited commercial impact; most research on the disease has focused on other commercially valuable cactus species, such as prickly pear cactus (*Opuntia* spp.) (Bousset *et al*., 2016; Yahia and Saenz, 2017; Kumar *et al*., 2018). Moreover, quadrant-based measurements can be a practical methodology for large areas because most of the cacti are over 30 years old and the population already overlaps; that is, if the symptoms start as small pustules, they can coalesce over time and appear as larger cankerous and necrotic areas (Feszt *et al*., 2009).

Our findings align with reported results, showing that both diagram-based and damage-type methods yielded similar responses, indicating that both can be used to estimate severity. The advantage is that, once the diagrams are established, this methodology is faster and more practical, particularly over larger areas or in denser populations. This study reports, for the first time, that *A. alternata* and *Phoma* spp. can cause necrotic spots on cardon cacti and proposes a quadrant model to estimate severity and incidence in a wild population, thereby improving conceptual strategies for preventive measures against fungal diseases in giant cardon cacti (*P. pringlei*) in B.C.S., Mexico, and worldwide.

Conclusions

Necrotic diseases are increasing in wild populations of cardon (*Pachycereus pringlei*) in Baja California Sur, Mexico, with the causal agents of the necrotic spot syndrome (NSS) not precisely identified and no effective control measures available. *Alternaria alternata* and *Phoma* spp. Were found as causal agents of NSS in the region. The both fungi can cause high levels of incidence and severity. Further study is needed to clarify the roles of other dematiaceous filamentous fungi that may be involved in necrotic diseases. The spread of the disease cannot be curbed, but appropriate epidemiological studies could help minimize the impact on these iconic desert specimens.

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ETHICS STATEMENT

The present study does not involve the participation of humans or animals.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTEREST

The authors declare no competing interest in this section.

FINANCING

All sources of funding for the reported research should be disclosed.

AUTHOR CONTRIBUTION

D.M.-H.; project administration; funding acquisition and molecular analyses. E.O.R.-P.; plant pathogenesis test, pathogenesis data interpretation. D.R.-J.; field methodology, analysis of severity values, and field supervisor. M.G.R.; formal analysis and revision of manuscript. B.L.-C.; software and review. R.J.H.-P.; writing—original draft, preparation and editing.

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