

Factors affecting the circular distribution of the leafless mistletoe *Tristerix aphyllus* (Loranthaceae) on the cactus *Echinopsis chilensis*

Factores que afectan la distribución circular del muérdago sin hojas *Tristerix aphyllus*
(Loranthaceae) sobre el cacto *Echinopsis chilensis*

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ABSTRACT

We describe the pattern of emergence of the holoparasitic mistletoe *Tristerix aphyllus* from its cactus host *Echinopsis chilensis* in a semiarid Chilean ecosystem. The observed circular distribution of the parasite inflorescence differed significantly from a uniform distribution based on a random process. We quantified the circular distribution of the seeds defecated on the cactus surface by *Mimus thenca*, the only bird responsible of seed dispersal. Our data did not support the idea of a directional seed deposition by the bird. To test the hypothesis that the observed circular distribution can be attributable to a differential seed survival due to microsite temperature variation, we infected cacti with seeds of *T. aphyllus* every 30° and quantified the temperature associated to each angle. Our results revealed that even though seeds located in angles with higher sun exposure had the lowest haustorial disk formation, this variation in mortality is not sufficient to explain the angular polarity observed in this species. Inspection of inflorescences of *T. aphyllus* that emerged 17 months after the experimental infection, revealed mean angular values indistinguishable from the natural circular distribution. Assessment of the anatomical structure at two opposing angles of the cactus revealed striking differences in epidermal constitution, such as a four-fold thicker epidermis in north than in south facing samples due to formation of highly lignified bark. We suggest that bark formation is likely the most important factor determining the biased circular distribution of *T. aphyllus*.

Key words: parasitic plant, inflorescence, seed deposition, bark formation, circular statistics.

RESUMEN

Describimos el patrón de emergencia del muérdago holoparásito *Tristerix aphyllus* desde su cacto hospedador *Echinopsis chilensis* en un ecosistema semiárido de Chile. La distribución circular de las inflorescencias del parásito difirió significativamente de una distribución uniforme basada en un proceso aleatorio. Cuantificamos la distribución circular de las semillas defecadas sobre la superficie del cacto por el mímido *Mimus thenca*, el único ave responsable de la dispersión del muérdago. Nuestros datos no sostuvieron la idea de una deposición de semillas direccional por parte del ave. Para someter a prueba la hipótesis que la distribución circular observada es atribuible a una sobrevivencia diferencial de las semillas debido a variación térmica entre micrositos, infectamos cactus con semillas de *T. aphyllus* cada 30° y evaluamos la temperatura asociada a cada ángulo. Aun cuando las semillas ubicadas en ángulos con mayor exposición solar presentaron la menor formación de disco haustorial, esta variación en mortalidad no fue suficiente para dar cuenta de la polaridad angular observada. No obstante, las inflorescencias de *T. aphyllus* que emergieron 17 meses después de la infección experimental, revelaron estadígrafos circulares indistinguibles de aquellos observados en la situación natural. La inspección de la estructura anatómica en dos ángulos opuestos de la cactácea reveló diferencias en la constitución de la epidermis, observándose un espesor en promedio cuatro veces mayor en las muestras orientadas hacia el norte que en las orientadas hacia el sur debido a la formación de corteza altamente lignificada. Sugerimos que la formación de corteza es probablemente el factor más importante en determinar la distribución circular sesgada de *T. aphyllus*.

Palabras clave: planta parásita, inflorescencia, deposición de semillas, formación de corteza, estadística circular.

INTRODUCTION

Establishment in suitable microhabitats is a critical step in determining the prevalence and abundance of parasites in host populations (see reviews in Esch et al. 1990, Poulin 1998). In parasitic plants, establishment involves two steps: i) attachment, defined as the adhesion of the haustorium to the host plant surface, and ii) penetration, defined as the process following attachment, where the peripheral cells of the radicle ingress to the host tissues (phloem or xylem) through the host cortex (Riopel & Timko 1995). Establishment is perhaps the most sensitive step from the parasitic life-cycle perspective (Norton & Carpenter 1998). It is known that variation in temperature, moisture, and light availability can dramatically reduce mistletoe seed survival (e.g., Lamont 1983, Sallé 1983, Boone et al. 1995), which in turn restrict the establishment of parasitic plants to a restricted set of within-host microhabitats (e.g., Norton et al. 1997, Yan & Reid 1995, Powell & Norton 1994). In this paper we assess the importance of the thermal environment for seed attachment and subsequent development in a holoparasitic mistletoe endemic to the arid zones of Chile.

Tristerix aphyllus (Loranthaceae) is a holoparasitic Chilean mistletoe that parasitizes only species of Cactaceae (Kuijt 1969, 1988). This unusual species has leaves reduced to minute scales and its red inflorescence emerges from the cactus surface. The only species responsible of consuming and dispersing the mistletoe seeds at the study site is the Chilean mockingbird *Mimus thenca* (Martinez del Río et al. 1995, Medel 2000). Once defecated by the bird, a reddish radicle emerges from a viscid seed, which often contacts and presses against the epidermis of cacti to form an haustorial disk. Once attached, narrow hairlike filaments pass through the stomata and the penetration of the cactus is effected (Mauseth et al. 1984, Mauseth et al. 1985). Attachment of the haustorium is not restricted to cacti but also occurs on a broad range of living and non-living substrates (Mauseth 1985). Within the cactus tissues, *T. aphyllus* constitutes an extensive system of endophytic strands leading in all directions (Kuijt 1969, Mauseth et al. 1984, Mauseth et al. 1985). The rest of the seedling remains on the outside of the cactus and dies after host penetration.

In this contribution we describe a striking microdistributional pattern of the inflorescences of *T. aphyllus* along the radial surface of the cactus *Echinopsis chilensis* (Colla) Friedr. et Rowl. (= *Trichocereus chilensis* B. et R.). At the same time, we evaluate the importance of biotic and

abiotic factors as the putative cause for the observed pattern. More specifically, we address the following questions: i) Does the emergence of the mistletoe inflorescences occur randomly from the cactus surface or exhibit an angular bias? ii) What is the importance of environmental temperature for the attachment process? iii) Can the circular distribution of inflorescences be explained by thermal differences between microhabitats? iv) What is the importance of cactus morphology in explaining the angular distribution of the mistletoe?

METHODS

Field procedure

The field study was conducted in the Reserva Nacional Las Chinchillas, Aucó (31°30'S, 71°06'W), approximately 300 km north Santiago. We measured the circular distribution of seeds and inflorescences from September to October 1997, using a mirror compass, and always setting the magnetic north to 0°. Because the mistletoe inflorescences are best represented by an arc on the perimeter of cacti, we recorded the minimum, bisecting, and maximum angle covered by the parasite in 118 cacti. Selected cacti averaged 1.85 m height (range: 0.23 – 2.55 m) and most mistletoes were located in the upper half of the cactus columns (1.28 m on the average, range: 0.11 – 2.26 m). Contrasts between the circular distribution of inflorescences and seed shadow were performed according to circular statistics procedures (Batschelet 1981, Fisher 1993).

Temperature analysis

To explore in more detail the factors involved in the circular pattern of the mistletoe, we quantified the angular variation in the daily temperature at the surface of one individual of *E. chilensis* 1.5 m above the ground. We measured simultaneously the temperature every 30° by setting thermocouples type T ($\pm 0.1\%$ accuracy) connected to a 12-channel scanning thermometer Digi-Sense. Temperature was recorded at the end of the seed dispersal period of *T. aphyllus* (December 1997), every 10 min from 7:00 to 21:00 h during three consecutive days.

To evaluate experimentally the role of temperature on the circular distribution of the mistletoe, in August 1998 we set 12 seeds per cactus at 1.5 m above ground. We placed one seed each 30° in 10 not parasitized host plants. Only seeds defecated by *M. thenca* and showing a radicle not

exceeding 3 mm long were selected for artificial infection. All seeds were oriented to ensure effective contact between the seed viscin and the host cuticle. We assessed seed survival and haustorial disk formation four months after manual infections. Both the subsequent emergence and the circular distribution of the 120 experimental infections were quantified in January 2000, 17 months after the initiation of experiment.

Anatomical analysis

To assess the importance of morphological barriers of cacti to the penetration or emergence of the mistletoe, we analyzed tissue samples of approximately 20 x 20 mm taken from five not parasitized individuals in a locality near Santiago. Tissue samples were taken 1.75 m above ground from north (0°) and south (180°) exposition. The depth of tissue sampling was approximately 10 mm. After each tissue sample was removed they were fixed immediately in F.A.A. (formalin-alcohol-acetic acid). Samples were analysed through Optical Microscope (OM) and Scanning Electron Microscopy (SEM). Samples were embedded in Paraplast, sliced in thin transversal sections (15

mm) and stained with safranin and fast-green for histological analysis at OM. SEM samples were dehydrated in an increasingly graded acetone series and dried from 100% acetone via CO₂ in a Polaron E3,000 critical point drying apparatus. Finally, they were coated with a gold layer 100 Å thick and viewed in the SEM Jeol JSM-25-SII. Measurements of surface thickness consisted on the linear distance from the outermost cell of the epidermis to the base of the hypodermis, adjacent to the chlorenchyma.

RESULTS AND DISCUSSION

Inflorescences described an arc of $132.6^\circ \pm 49.9^\circ$ on the surface of *E. chilensis* (mean vector \pm circular SD, range: $36.0^\circ - 267.0^\circ$, N = 118), and the circular distribution of the bisecting angles differed significantly from a uniform distribution (mean vector \pm circular SD, $118.1^\circ \pm 42.0^\circ$, range: $50.7^\circ - 186.3^\circ$, Rayleigh test for uniformity, $P < 0.001$). This pattern implies a conspicuous polarity in the circular distribution of *T. aphyllus* in the direction SE that can not be explained by a random process (Fig. 1). We assessed the seed shadow of the mistletoe on cacti as a potential factor

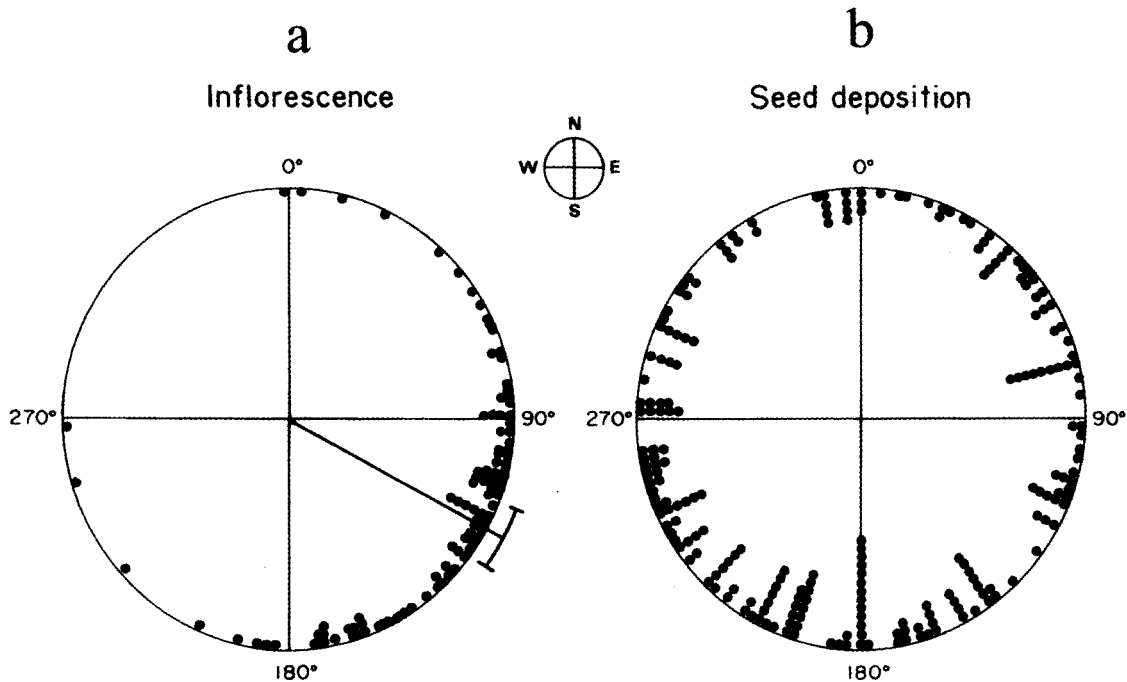


Fig. 1. Circular distribution of the bisecting angle of inflorescences of *Tristerix aphyllus* on *Echinopsis chilensis* (a), and circular distribution of the seed deposition made by *Mimus thenca* on cacti (b). The mean vector and circular deviation in the inflorescence data are indicated.

Distribución circular del ángulo bisectriz de las inflorescencias de *Tristerix aphyllus* sobre *Echinopsis chilensis* (a), y distribución circular de la deposición de semillas efectuada por *Mimus thenca* sobre los cactus. (b) Se indica el vector promedio y la desviación circular en los datos de inflorescencia.

responsible for the angular polarity of inflorescences. Because seeds remain adhered to cacti once defecated by *M. thenca*, it was possible to estimate the circular distribution of the seed shadow by recording the exact angle at which every seed was observed on the host (mean vector of the seed shadow \pm circular SD, $197.6^\circ \pm 104.2^\circ$). The inflorescence distribution was more concentrated than the seed shadow (2.49 and 0.39, respectively), and comparison of the mean vectors revealed significant differences between the two distributions (Watson F-test for two circular means, $F = 59.76$, $df = 337$, $P < 0.001$, Fig. 1). This result indicates that the seed shadow does not necessarily translate into the observed emergence of the inflorescences, and consequently can not be considered as responsible for the polarity in the circular distribution of the mistletoe.

Like non-parasitic plants, germination and development of mistletoes require permissive temperatures (Boone et al. 1995) and the optimum temperature for germination and radicle elongation varies depending on the environment (Lamont 1983). In the study site, the mean temperature around the cactus ranged from 25.9 - 29.5 °C ($\Delta T = 4.6$ °C), and the highest value was observed at 270° (Fig. 2). The maximum temperature ranged from 36.6 - 47.9 °C ($\Delta T = 11.3$ °C), and the highest value also occurred at 270°. The minimum temperature ranged from 12.4 - 14.0 °C ($\Delta T = 1.6$ °C). Because the greatest temperature variation was observed in maximum temperatures, it is reasonable to consider this as one of the likely constraining factors for mistletoe seed survival.

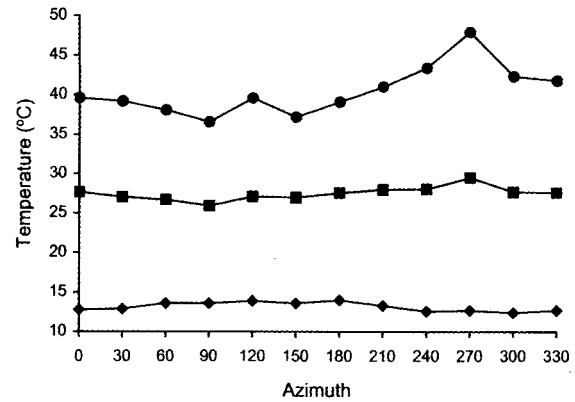


Fig. 2. Descriptors of the maximum (●), mean (■), and minimum (◆) temperature for 12 thermocouples set up at intervals of 30° on the surface of *Echinopsis chilensis*.

Descriptoros de la temperatura máxima (●), media (■), y mínima (◆) para 12 termocuplas ubicadas a intervalos de 30° sobre la superficie de *Echinopsis chilensis*.

Results from the experimental infection revealed that seeds located between 270 - 330° had a lower probability of successful infection as shown by haustorial disk formation (Fig. 3). Overall, 69.2 % of the 120 experimental seeds survived to form haustorial disk (t-test for dependent samples, $t = 4.27$, $df = 11$, $P = 0.001$). However, disk formation was not always followed by a subsequent emergence of the mistletoe from the cactus cuticle. Only 15.8 % of the infected cacti showed inflorescence emergence 17 months after experimental infection (Fig. 3), a relatively low fraction

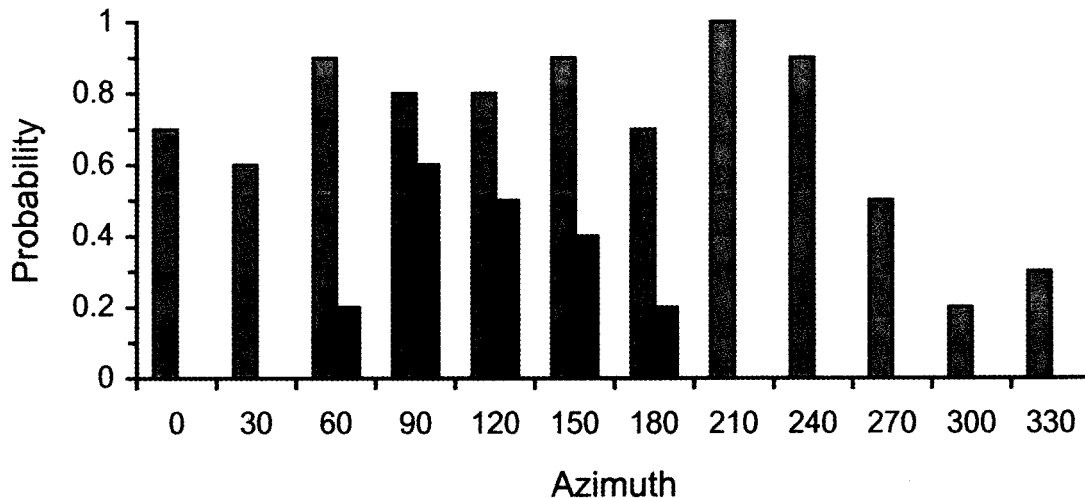


Fig. 3. Probability of haustorial disk formation (grey bars) and subsequent emergence of inflorescences (black bars) of *Tristerix aphyllus* from the experimental infection on *Echinopsis chilensis*.

Probabilidad de formación de disco haustorial (barras grises) y subsecuente emergencia de inflorescencias (barras negras) de *Tristerix aphyllus* a partir de las infecciones experimentales sobre *Echinopsis chilensis*.

in comparison to the total seeds that developed haustorial disk (19 out of 83 seeds) (t-test for dependent samples, $t = 7.09$, $df = 11$, $P < 0.001$). Emergence of new inflorescences occurred in the range $60^\circ - 180^\circ$ (Fig. 3), which fits well to the natural circular distribution of emergence (range: $50.7^\circ - 186.3^\circ$). Comparisons of the minimum, mean and maximum angles of the natural inflorescences with those emerged from experimental infected cacti did not reveal significant differences (Table 1). Overall, these results indicate that temperature, although important for seed survival and haustorial disk formation, is not sufficient to explain the emergence of new inflorescences and thus the angular polarity of *T. aphyllus* observed at this level.

Our results indicate that seed deposition and temperature can not unequivocally be identified as responsible for the angular pattern of the inflorescences. Even though temperature seems to be an important abiotic factor influencing the emergence of *T. aphyllus* from the cactus tissue, other factors need to be assessed for a better understanding of the angular polarity of this species. For instance, anatomical analysis revealed striking differences in the surface thickness of cactus depending from the azimuth (see also Evans et al. 1994c). South facing samples (180°) consist on a smooth thin epidermis that cover a thick hypodermis made of six to eight layers of cells with extremely thick walls (Fig. 4a). Above the epidermis there is a profusion of epicuticular waxes that originate in the epidermic cells, leaving stomata deeply sunken under the surface. Although stomata are located at the surface, cuticularized stomatal canals pass from the stomata through the hypodermis to the assimilatory tissues beneath. Interestingly, north facing samples (0°) consist on several layers of highly lignified periderm, which originates from cellular divisions in the epidermal cells (Fig. 4b). Sequential analysis of transversal thin sections of tissue samples indicated that, unlike samples from the south orientation, several overlapping layers of periderm clearly obstruct stomata located in the epidermis, inhibiting their function in gas exchange between inside and outside of the stem. Comparison of the stem thickness between samples revealed a four-fold higher thickness in north than in south samples (mean (mm) \pm SD, north: 1539.0 ± 508.7 ; south: 374.9 ± 80.0 , $n = 5$, paired t-test, $t = 5.05$, $P < 0.001$). Because infection occurs when hairlike filaments of *T. aphyllus* pass through the stomata of cacti (Mauseth 1985, Mauseth et al. 1984, 1985), the asymmetrical formation of peridermis seems to be the most important factor responsible of the bias in the circular distribution of *T.*

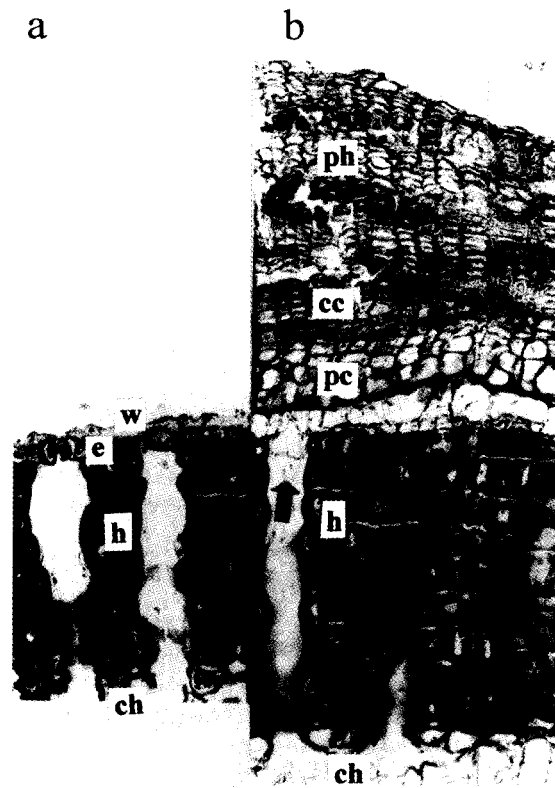


Fig. 4. SEM microphotographies showing transverse sections of stems at the level of the surface of *Echinopsis chilensis*. a) South facing sample showing an epidermis of two or three layers covered with a great profusion of epicuticular waxes with sunken stomata. Under the epidermis there is a pluristratified hypodermis of very thick walls, interrupted by long sub-stomatic chambers which end in the chlorenchyma, x 50. b) North facing sample showing a barking surface with several overlapped layers of periderm obstructing stomata (shown by an arrow), x 50. (e: epidermis, w: epicuticular waxes, h: hypodermis, ch: chlorenchyma, cc: cork cambium, ph: phellem, pc: parenchymatic cells).

Microfotografías de microscopía electrónica indicando secciones transversales de la superficie de *E. chilensis*. a) Muestra de exposición sur mostrando una epidermis de dos - tres capas cubiertas con ceras epicuticulares con estomas hundidos. Bajo la epidermis hay una hipodermis pluriestratificada de paredes muy gruesas, las cuales son interrumpidas por largas cámaras sub-estomáticas que terminan en el clorénquima, x 50. b) Muestra de exposición norte mostrando una superficie de corteza con varias capas sobrepuestas de peridermis que obstruyen los estomas (indicado por flecha), x 50. (e: epidermis, w: ceras epicuticulares, h: hipodermis, ch: clorénquima, cc: felógeno, ph: peridermis, pc: células parenquimáticas).

TABLE I

Results from contrasts of the mean vectors (SD) of inflorescences of *Tristerix aphyllus* in natural and field experimental conditions (df = 125 for all comparisons)

Resultados de contrastes de los vectores promedios (SD) de las inflorescencias de *Tristerix aphyllus* en condiciones naturales y experimentales de terreno (gl = 125 para todas las comparaciones)

	Minimum angle	Bisecting angle	Maximum angle
Natural	50.73° (52.64°)	118.05° (41.97°)	186.28° (44.20°)
Experimental	44.80° (30.92°)	105.83° (26.53°)	167.93° (32.11°)
F	0.11	0.73	1.42
P	0.74	0.39	0.24

aphyllus. There is evidence that establishment and haustorial connections in some hemiparasitic mistletoes infecting tree species tend to relate inversely with host bark thickness (e.g., Sargent 1995, Norton & Ladley 1998). We hypothesize that uneven bark formation in the surface of *E. chilensis* represents a critical physical barrier for the conductivity of the haustorium of *T. aphyllus*. However, bark formation in the north facing epidermis is not restricted to *E. chilensis* as revealed by detailed studies done in several cactus species from the northern and southern hemispheres (see Evans et al. 1994a, b, c). These authors suggested that the causal agent of barking relates with sun exposure and UV irradiance. The extent to which bark formation in *E. chilensis* is adaptive to prevent parasitism or represent an incidental effect to environmental conditions with no implication for the host-parasite coevolution remains to be tested in future studies.

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