

Variation of the architectural phenotype of *Tristerix aphyllus* in central Chile

Variación del fenotipo arquitectural de *Tristerix aphyllus* en Chile central

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ABSTRACT

We analyze the morphological variation of the holoparasitic mistletoe *Tristerix aphyllus* in 20 populations of central Chile by focusing upon 8 qualitative characters that represent different architectural attributes of the parasite. Our results show that specific characters present a low congruent variation across populations, which suggests a low integration of the architectural phenotype. Similarly, 44 different combinations of traits were identified, and their prevalence at each population was indistinguishable from that occasioned by a random process. Characters did not contribute equally to the overall architectural variation. Sympodial growth pattern, tetrapodism, single branch, and closed branch were the most important traits in contributing to architectural variation across populations. The disintegrated architectural phenotype indicates absence of a unique architectural design in this species. Rather, the random prevalence of character combinations suggests that the geographical pattern may be better described as a "random patchwork". On the base of these antecedents, and considering the leafless holoparasitic biology of *T. aphyllus* we suggest that non adaptive architecture prevails in this species.

Key words: mistletoe, parasite, host, Loranthaceae, Mediterranean habitat.

RESUMEN

Analizamos la variación morfológica del muérdago holoparásito *Tristerix aphyllus* en 20 poblaciones de Chile central. Focalizamos en 8 caracteres cualitativos los cuales representan diferentes atributos arquitecturales del parásito. Los caracteres específicos presentan una baja variación congruente entre poblaciones, lo cual sugiere una baja integración del fenotipo arquitectural. De igual forma, se identificaron 44 combinaciones distintas de caracteres y su prevalencia en las poblaciones fue indistinguible de una ocasionada por azar. Los caracteres no contribuyeron de igual manera a la variación arquitectural. Patrón de crecimiento simpodial, tetrapodismo, rama simple, y rama cerrada fueron las rasgos más importantes en contribuir a la variación total. La desintegración fenotípica documentada en este estudio sugiere ausencia de un único diseño arquitectural en esta especie. Por el contrario, la prevalencia aleatoria de combinaciones de caracteres en las poblaciones sugiere que el patrón geográfico podría ser mejor descrito como un "mosaico aleatorio". Sobre la base de estos antecedentes, y considerando la biología holoparásita sin hojas de *T. aphyllus*, sugerimos que los rasgos arquitecturales que prevalecen en esta especie no conllevan valor adaptativo.

Palabras clave: muérdago, parásito, hospedador, Loranthaceae, hábitat mediterráneo.

INTRODUCTION

Architecture is an important but often neglected component of the plant phenotype. Architectural design tend to be related with nutrient absorption and light interception (Küppers 1989, Room et al. 1994), and certain combinations of traits have provided to be optimal for increased nutrient uptake (e.g., Lovell & Lovell 1985, Küppers 1989). In spite of the potential importance of architecture to reveal the way environment

constraints plant form and development, most studies dealing with plant architecture have been conducted on non-parasitic plants where vegetative and reproductive axes are integrated into the same structure (Hallé et al. 1978, Kuuluvainen & Pukkala 1991). Situation with holoparasitic plants is less clear because hosts can potentially provide sufficient nutrients to make unnecessary any single optimal architectural design in parasites. Hosts represent the more immediate surrounding for the parasite (Herrera

1988a, Hutchings & de Kroon 1994), and phenotypic evolution of some hemiparasitic species has been demonstrated to be influenced by host species (e.g., Atsatt 1970a, Atsatt & Guldberg 1978). In spite of, studies dealing with the architecture of holoparasitic plants are lacking in the literature.

This paper focuses on the architectural design of *Tristerix aphyllus*, a Chilean endemic holoparasitic mistletoe that parasitizes a total of 17 species of cacti in the Chilean arid and semiarid zones (Follman & Mahú 1964). Research on the architecture of *T. aphyllus* is pertinent because several anatomic and physiological properties make this species an unusual mistletoe that depends entirely on the host for growth and reproduction (Kuijt 1969). For example, unlike most Loranthaceae, *T. aphyllus* has leaves reduced to minute scales with only the reproductive structure emerging from the parasitized cacti. The rest of vegetative portion exists as an endophyte within the tissues of the cacti (Mauseth 1990). These characteristics have been interpreted as anatomic adaptations to reduce potential excessive water loss in Chilean arid zones (Mauseth 1985, 1991). Although the mechanism of host entry and the associated anatomical characteristics of *T. aphyllus* have been extensively studied by Mauseth et al. (1984, 1985), the morphology of its reproductive portion has not received similar attention. The only reference indicates stems of its external portion are reddish and 5-20 cm long (Hoffmann 1978). In an extensive review of the genus, Kuijt (1988) referred to the morphology of *Tristerix* as "The branching pattern of all species seems to be basically the same, although the situation in *T. aphyllus* needs more careful study." (p. 6).

The aim of this paper is to assess the extent to which the architectural phenotype of a holoparasitic mistletoe varies uniformly across populations as expected for an optimal architectural design. This study addresses the following questions. (1) Are populations of *T. aphyllus* homogeneous in architectural traits?, (2) Do architectural traits vary independently across populations or are they organized into coordinate varying subsets? Responses to these questions are related to the more general one, (3) does architecture of

T. aphyllus vary uniformly or does it represent a random patchwork with traits combining randomly across populations?

MATERIALS AND METHODS

We sampled 20 populations of *T. aphyllus* in central Chile. We selected 10 populations from the Reserva Nacional Las Chinchillas ($31^{\circ} 30' S$, $71^{\circ} 06' W$), located 17 km north Illapel. Other 10 populations were selected around Til-Til ($33^{\circ} 28' S$, $70^{\circ} 52' W$), located approximately 60 km north Santiago. Sampling consisted in collecting 40 mistletoes each from a different individual of the cactus *Echinopsis chilensis* per population, taking care of removing completely the reproductive portion of mistletoes. Samples were carried to the lab for analyses and examined with a binocular microscope. We examined a total of 800 individuals for architectural traits. All sampling was conducted during March-May 1994, during the flowering season of *T. aphyllus*.

We focused upon eight qualitative characters related to the architectural design of the mistletoe (Figure 1) (Prusinkiewicz & Lindenmayer 1990): 1) Monopodial pattern of growth, that refers to the situation when the main apex continues growing but all lateral apices stop growing. 2) Sympodial pattern of growth, that occurs when the principal axe is occupied by a lateral branch that gets apical dominance. 3) Tetrapodic branch, that refers to the formation of a verticile with four branches with shortened internode distances. 4) Single branch, that refers to the formation of a unique lateral branch along the principal axis. 5) Double branch, that refers to the formation of two lateral branches with a minimal internode distance. 6) Closed branch, that refers to a relative shortening of the internode distance among alternate pairs of branches when branching is opposite along the principal axis. 7) Opposite branch, that refers to the formation of lateral branches in an opposite form along the principal axis. 8) Alternate branch, that refers to the formation of lateral branches in an alternate form along the principal axis. The first two characters represent a unit of analysis somewhat different from the

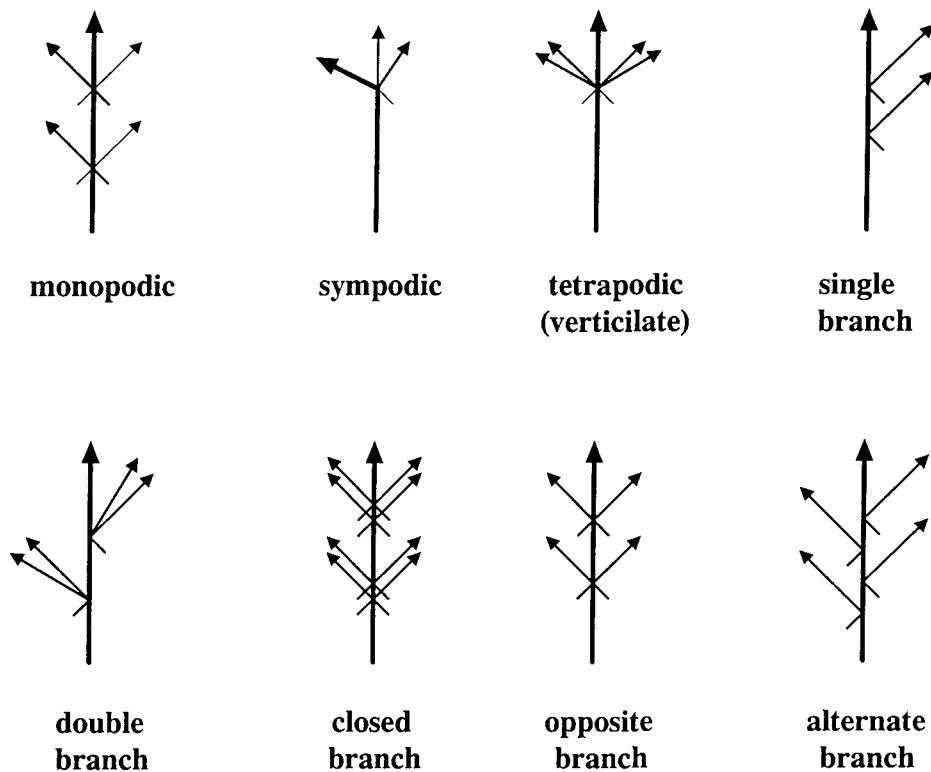


Fig. 1: Graphical description of the architectural characters of *T. aphyllus* used in this study.

Descripción gráfica de los caracteres arquitecturales de *T. aphyllus* usados en este estudio.

remaining traits because of their higher importance to the overall pattern of growth. Because abortion of a meristem can potentially originate a monopodial and/or a sympodial pattern (Callaghan et al. 1986, Mueller 1988), we searched for the two characters at each individual.

Populations examined comprised two distant localities (10 populations from Las Chinchillas and 10 around Til-Til). Our first analysis consisted in assessing for potential differences in architectural traits among the two groups of populations. We calculated the percentage of mistletoes exhibiting each character at each population and assessed for global differences among the two localities by discriminant analysis on the multivariate architectural phenotype (Hair et al. 1992). We next evaluated if pairs of traits exhibited congruent variation across populations. We constructed correlation matrixes using every character pair at each of the two localities and tested for heterogeneity of correlation coefficients among the two situations. We

applied a test for homogeneity to each pair of coefficients by using the Hotteling's Z^2 transformation after applying the Bonferroni criterium to correct for the 28 multiple contrasts (Rice 1989, Sokal & Rohlf 1995).

Previous analyses revealed that none of the 800 individuals examined exhibited the eight characters simultaneously, but 44 different trait combinations were detected. In order to know the traits that contribute most to the total architectural variation, we classified every individual according to its particular trait combination and proceeded to calculate an index of population architectural variation. We used the inverse of Simpson's index, $B = 1/\sum \pi_i^2$, where π_i represents the population frequency of individuals with architectural combination i . With this classification we evaluated the contribution of each trait to the total architectural variability by using stepwise regression with no variables forced into the models and P values for partial F tests of 0.05 to enter and 0.10 to remove. Because characters were present in

more than one combination, they may not affect independently the architectural variation of *T. aphyllus*. To evaluate such possibility, we tested for correlated causes of characters on architectural variability, B, by means of path analysis.

In order to assess for coordinate variation of subsets of characters across populations we calculated the similarity in the trait combinations among pairs of populations by means of the Jaccard's similarity index. Because similarity may not differ from a random sampling of combinations, we constructed a bootstrap algorithm (Efron 1981, Manly 1991), that consisted in the random assignation of trait combinations to each population. The selected combination was returned each time to the original database up to completing the real number of combinations in the population. In this way, it was possible to compare the observed distribution of trait combinations in the 20 populations with a distribution based on a random sampling process. Excepting the randomization procedure, all analyses were carried out by using SYSTAT statistical package (Wilkinson 1992).

RESULTS AND DISCUSSION

We detected significant differences in four of the eight characters examined for variation, which tended to be more frequent in the study sites near Til-Til (Table 1). When all characters are considered together, the multivariate architectural space was affected by locality (Wilks' lambda = 0.124,

$F(8,11) = 9.72$, $P = 0.001$, MANOVA). Only the first of the eight canonical axes revealed significant discrimination (83.2% of the differences among localities). The different signs of some coefficients of the canonical discriminant function indicate that antagonistic characters related to the apical dominance processes (monopodism and sympodium) and antagonistic characters related to formation of lateral branches (opposite and alternate branch) were important in discriminating among localities (Table 2). However, comparison of the structure of character correlation matrixes did not reveal significant differences among localities. This suggests that the determinants of architectural integration in *T. aphyllus* have not undergone significant differentiation among localities.

Most architectural characters tended to vary independently across populations excepting six pairs that associated congruently in populations near Til-Til. None of such pairs exhibited significant association in Las Chinchillas. The only consistent trend in trait covariation was the pair monopodial growth-opposite branch, which showed a significant positive relationship in populations of the two localities (Table 3). When the two correlation matrixes were tested for homogeneity, the null hypothesis of homogeneity of correlation coefficients could not be rejected, which imply that architectural characters do not associate in different ways in the two localities. Consequently, it is possible to conclude that the components of the architectural phenotype of *T. aphyllus* do not present congruent

TABLE I
Frequency of architectural characters of *T. aphyllus* in 20 populations of
Las Chinchillas and Til-Til. Figures indicate mean \pm 2 SE
Frecuencia de caracteres arquitecturales de *T. aphyllus* en 20 poblaciones de Las Chinchillas y Til-Til.
Los valores indican media \pm 2 EE

Character	Las Chinchillas	Til-Til	df	F	P
monopodism	1.00 \pm 0.00	0.99 \pm 0.01	1,18	1.0	0.331
sympodium	0.15 \pm 0.04	0.24 \pm 0.08	1,18	3.06	0.097
tetrapodism	0.03 \pm 0.02	0.06 \pm 0.03	1,18	2.44	0.135
single branch	0.06 \pm 0.03	0.16 \pm 0.02	1,18	22.76	< 0.001
double branch	0.03 \pm 0.01	0.11 \pm 0.02	1,18	31.59	< 0.001
closed branch	0.03 \pm 0.03	0.13 \pm 0.06	1,18	11.13	0.004
opposite branch	0.97 \pm 0.02	0.99 \pm 0.01	1,18	9.11	0.007
alternate branch	0.65 \pm 0.08	0.59 \pm 0.08	1,18	0.96	0.339

TABLE 2

Coefficients of the standardized canonical discriminant function in the multivariate comparison of architecture of *T. aphyllus* among localities

Coeficientes de la función discriminante canónica estandarizada en el contraste multivariado de la arquitectura de *T. aphyllus* entre localidades

Character	First canonical axe
Monopodium	-0.050
Sympodium	0.235
Tetrapodium	0.163
Single branch	0.462
Double branch	0.387
Closed branch	0.482
Opposite branch	0.366
Alternate branch	-0.135

variation but tend to vary independently across populations.

When combinations of characters rather than specific traits were analyzed, a total of 44 different combinations was observed in the 20 populations which occasioned values of variability, B, ranging from 2.18 to 8.69. Contribution of specific characters to architectural variation was assessed by stepwise regression. Four characters were selected as

contributing significantly to architectural variation (first entry: sympodial growth $P < 0.001$; second entry: tetrapodism $P < 0.001$; third entry: closed branch $P < 0.05$; fourth entry: single branch $P < 0.05$). The analysis of independent causes measured by Pearson's correlation analysis revealed significant coefficients when each trait was correlated with B. Path analysis revealed correlated correlation coefficients similar to Pearson's independent coefficients (sympodial growth = 0.853, tetrapodism = 0.755, single branch = 0.683, closed branch = 0.603). This result implies that correlation with B is occasioned not only by the direct contribution of individual characters but also by interactions among them. This is graphically depicted in Figure 2.

Similarity values among populations in the types of characters combinations were low (mean ± 2 SE: 0.14 ± 0.21), and comparison with values expected from a random process revealed non significant differences (Student's test, $P > 0.16$). This result implies that the subsets of architectural characters do not tend to vary coordinately across populations which further confirms the previous finding of absence of congruent variation of

TABLE 3

Correlation matrix for congruent variation of characters of *T. aphyllus*. LC = Las Chinchillas, TT = Til-Til, Mono = monopodium, Symp = Sympodium, Tetr = tetrapodium, Sbra = single branch, Dbra = double branch, Cbra = closed branch, Obra = opposite branch.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Matriz de correlación para variación congruente de caracteres de *T. aphyllus*. LC = Las Chinchillas, TT = Til-Til, Mono = monopodismo, Symp = simpodismo, Tetr = tetrapodismo, Sbra = rama simple, Dbra = rama doble, Cbra = rama cerrada, Obra = rama opuesta. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

	Mono	Symp	Tetr	Sbra	Dbra	Cbra	Obra
Sympodium	LC	0.56	-0.16	-	-	-	-
	TT	-0.16	-	-	-	-	-
Tetrapodium	LC	-0.41	-0.05	-	-	-	-
	TT	-0.70*	0.54	-	-	-	-
Single branch	LC	-0.19	0.23	-0.05	-	-	-
	TT	-0.67*	0.56	0.64 ⁸	-	-	-
Double branch	LC	-0.07	-0.50	0.06	-0.17	-	-
	TT	-0.36	0.26	0.49	0.70*	-	-
Closed branch	LC	0.44	0.31	-0.26	0.37	-0.30	-
	TT	-0.05	0.27	0.29	-0.19	-0.41	-
Opposite branch	LC	0.82**	0.37	-0.52	0	-0.25	0.49
	TT	1.00***	-0.16	-0.70*	-0.67*	-0.36	-0.05
Alternate branch	LC	0.41	0.19	-0.07	0.10	0.33	0.37
	TT	0.20	0.07	0.01	0.11	-0.06	0.31
						-0.03	0.20

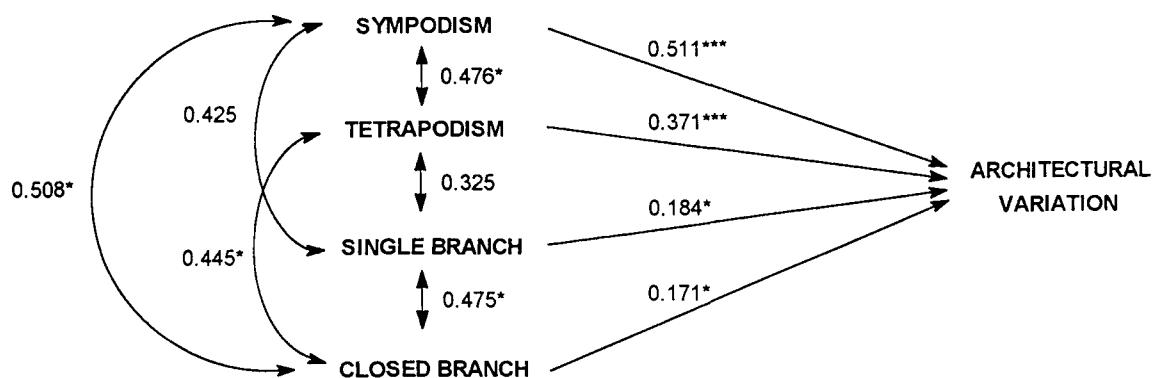


Fig. 2: Path diagram illustrating the architectural characters that correlate with the overall architectural variation. Figures over straight lines pointing to the architectural variation indicate standard partial regression coefficients. Figures over lines connecting architectural characters indicate Pearson's correlation coefficients. * $P < 0.05$, *** $P < 0.001$.

Diagrama de vías que ilustra los caracteres arquitecturales que se correlacionan con la variación arquitectural. Los valores sobre las líneas rectas apuntando hacia variación arquitectural indican coeficientes de regresión parcial estándar. Los valores sobre las líneas que conectan los caracteres arquitecturales indican coeficientes de correlación de Pearson. * $P < 0.05$, *** $P < 0.001$.

specific characters across populations. On the base of these results it is possible to conclude that different architectural phenotypes of *T. aphyllus* appear randomly across populations.

The absence of covariation in individual characters at each locality and the high number of different character combinations found among populations suggests that the pattern of architectural morphology may be better described as a "random patchwork", with frequencies of characters and character combinations occurring randomly across populations. The high architectural diversity found in this study suggests absence of a single design for *T. aphyllus*, thus questioning the existence of adaptive architecture in this species. Mechanisms responsible for the high architectural variability of *T. aphyllus* are unknown. Biotic factors such as the identity of pollinators and fruit dispersers species may contribute to increase variability among plant populations. However, *T. aphyllus* is pollinated almost exclusively by the hummingbird *Sephanoides galleritus* and its fruits are dispersed uniquely by the Chilean mockingbird *Mimus thenca*. Although changes in the density of pollinator and disperser species may eventually affect the architecture of this holoparasite, it is unlikely that architectural variation among populations is occasioned by changes in

pollinator and disperser assemblages across populations.

The high architectural variation documented in this paper may be attributed, at least in part, to the condition of holoparasite favoring phenotypical canalization by hosts (Atsatt 1970a). Independent variation among populations may stem from genetic differences, host age differences and variation in host physiological and nutrient status, or most likely a combination of these. For instance, high phenotypical variation in morphological and reproductive traits is a conspicuous feature of hemiparasites (Smith 1963, Bobear 1969, Atsatt 1970a,b, Atsatt & Guldberg 1978, Herrera 1988b) that tend to be correlated with increased genetic diversity (Atsatt 1970a, Atsatt & Strong 1970, Atsatt & Guldberg 1978). Although the genetic basis of architectural design in *T. aphyllus* is unknown, the factors that determine the phenotypical variation in this species are probably different from those concerning land autotrophic or even hemiparasitic plants (Atsatt 1983, Aparicio 1993).

The high diversity of architectural phenotypes within populations suggests that the physiological and nutrient status of hosts may be more important than external environmental factors in determining the parasite phenotype. It is possible that nutrient absorption from external sources and light

interception do not represent important constraints on its architecture at present. Perhaps the most parsimonious explanation for the overall high degree of phenotypical variation relies in the peculiar leafless structure of the plant and the high differentiation among vegetative and reproductive portions. Because the external portion of *T. aphyllus* consists uniquely of leafless reproductive axes, it is quite possible that actual architecture bears no direct relationship with light and carbon capture thus lacking adaptive value.

Architectural optimization in plants relies on the assumption that resource acquisition from external sources is constrained by organismal design (Küppers 1989, Room et al. 1994). It is possible that no architectural optimum is needed in *T. aphyllus* in the light of the constant and predictable source of water and nutrients provided by cacti hosts. Our results allow us to postulate that architectural design in the holoparasitic *T. aphyllus* is not related to light and nutrient capture. Consequently, it is possible that most loci in this species behave neutral to architecture evolution and non adaptive architectural variation prevails across populations.

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LITERATURE CITED

- APARICIO A (1993) Sex-determining and floating translocation complexes in *Viscum cruciatum* Sieber ex Boiss. (Viscaceae) in southern Spain. Some evolutionary and ecological comments. Botanical Journal of the Linnean Society 111: 359-369.
- ATSATT PR (1970a) Hemiparasitic flowering plants: phenotypic canalization by hosts. Nature 225: 1161-1163.
- ATSATT PR (1970b) The population biology of annual grassland hemiparasites. II. Reproductive patterns in *Orthocarpus*. Evolution 24: 598-612.
- ATSATT PR (1983) Mistletoe leaf shape: a host morphogen hypothesis. In: Calder M & P Bernhardt (eds) The biology of mistletoes. Academic Press, Sydney.
- ATSATT PR & DE STRONG (1970) The population biology of annual grassland hemiparasites. I. The host environment. Evolution 24: 278-291.
- ATSATT PR & LD GULDBERG (1978) Host influence on floral variability in *Orthocarpus densiflorus* (Scrophulariaceae). Plant Systematics and Evolution 129: 167-176.
- BOBEAR JB (1969) An analysis of populations of Irish *Euphrasia*. Watsonia 7: 68-91.
- CALLAGHAN TV, AD HEADLEY, BM SVENSSON, L LIXIAN, JA LEE & DK LINDLEY (1986) Modular growth and function in the vascular cryptogam *Lycopodium annotinum*. Proceedings of the Royal Society of London Ser. B 228: 195-206.
- EFRON B (1981) Non parametric standard errors and confidence intervals. Canadian Journal of Statistics 9: 139-172.
- FOLLMAN G & M MAHU (1964) Las plantas huéspedes de "Phrygilanthus aphyllus" (Miers) Eichl. Boletín de la Universidad de Chile, Ciencias 7: 39-41.
- HAIR JF, RE ANDERSON, RL TATHAM & WC BLACK (1992) Multivariate data analysis. Third edition. MacMillan and Co., New York.
- HALLE F, RAA OLDMAN & PB TOMLINSON (1978) Tropical trees and forests: an architectural analysis. Springer-Verlag, Berlin.
- HERRERA CM (1988a) Plant size, spacing patterns, and host-plant selection in *Osyris quadripartita*, a hemiparasitic dioecious shrub. Journal of Ecology 76: 995-1006.
- HERRERA CM (1988b) The fruiting ecology of *Osyris quadripartita*: individual variation and evolutionary potential. Ecology 69: 233-249.
- HOFFMANN A (1978) Flora silvestre de Chile: zona central. Ediciones Fundación Claudio Gay, Santiago, Chile.
- HUTCHINGS MJ & H DE KROON (1994) Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25: 159-238.
- KUIJT J (1969) The biology of parasitic flowering plants. University of California Press, Berkeley.
- KUIJT J (1988) Revision of *Tristerix* (Loranthaceae). Systematic Botany Monographs 19: 1-61.
- KÜPPERS M (1989) Ecological significance of above-ground architectural patterns in woody plants: a question of cost-benefit relationships. Trends in Ecology and Evolution 4: 375-379.
- KUULUVAINEN T & T PUUKALA (1991) Interaction between canopy architecture and photosynthetically active direct radiation at different latitudes: simulation experiments and their ecological implications. In: Edelin C (ed) L'arbre: biologie et développement. Naturalia Montpeliensis A7, Montpellier.
- LOVELL PH & PJ LOVELL (1985) The importance of plant form as a determining factor in competition and habitat exploitation. In: White J (ed) Studies on plant demography: a festschrift for John L. Harper. Academic Press, London.
- MANLY BFJ (1991) Randomization and Monte Carlo methods in biology. Chapman & Hall, London.
- MAUSETH JD (1985) Relations between *Trichocereus chilensis* and the holoparasite *Tristerix aphyllus*. Medio Ambiente 7: 39-44.
- MAUSETH JD (1990) Morphogenesis in a highly reduced plant: the endophyte of *Tristerix aphyllus* (Loranthaceae). Botanical Gazette 151: 348-353.

- MAUSETH JD (1991) Botany: an introduction to plant botany. Saunders College Publishing, Florida.
- MAUSETH JD, G MONTENEGRO & AM WALCKOWIAK (1984) Studies of the holoparasite *Tristerix aphyllus* (Loranthaceae) infecting *Trichocereus chilensis* (Cactaceae). Canadian Journal of Botany 62: 847-857.
- MAUSETH JD, G MONTENEGRO & AM WALCKOWIAK (1985) Host infection and flower formation by the parasite *Tristerix aphyllus* (Loranthaceae). Canadian Journal of Botany 63: 567-581.
- MUELLER RJ (1988) Shoot tip abortion and sympodial branch reorientation in *Brownea ariza* (Leguminosae). American Journal of Botany 75: 391-400.
- PRUSINKIEWICZ P & A LINDENMAYER (1990) The algorithmic beauty of plants. Springer-Verlag, New York.
- RICE WR (1989) Analysing tables of statistical tests. Evolution 43: 223-225.
- ROOM PM, L MAILLETTE & JS HANAN (1994) Module and metamer dynamics and virtual plants. Advances in Ecological Research 25: 105-157.
- SMITH AJE (1963) Variation in *Melampyrum pratense* L. Watsonia 5: 336-367.
- SOKAL RR & FJ ROHLF (1995) Biometry: the principles and practice of statistics in biological research. Third edition. Freeman and Co., New York.
- WILKINSON L (1992) SYSTAT for Windows: Statistics. 5th edition. Evanston, Illinois.