Mediación del hospedero sobre la interacción del muérdago *Tristerix verticillatus* (Loranthaceae) con su ensamble de polinizadores y consecuente intercambio de polen

Tesis

Entregada A La

Universidad De Chile

En Cumplimiento Parcial De Los Requisitos

Para Optar Al Grado De

Doctor en Ciencias con mención en Ecología y Biología Evolutiva

Facultad De Ciencias

Por

Maria Alejandra Troncoso Joffre

Setiembre, 2012

Directores de Tesis: Dr. Hermann M. Niemeyer & Dr. Christian C. Figueroa

FACULTAD DE CIENCIAS
UNIVERSIDAD DE CHILE
INFORME DE APROBACIÓN
TESIS DE DOCTORADO

Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la Tesis de Doctorado presentada por el candidato

MARIA ALEJANDRA TRONCOSO JOFFRE

Ha sido aprobada por la Comisión de Evaluación de la tesis como requisito para optar al grado de Doctor en Ciencias con mención en Biología, en el examen de Defensa de Tesis rendida el 10/05/2012

Director de Tesis:

Dr. Hermann M. Niemeyer ....

Co-director de Tesis:

Dr. Christian C. Figueroa ....

Comisión de Evaluación de Tesis:

Dr. Rodrigo Medel ....

Dr. Ramiro Bustamante ....

Dr. Sylvain Faugeron ....

Dr. Rodrigo Ramos ....

Dr. Daniel Frías ....

A Renzo
mi cómplice, mi amor y todo
A Nataniel
nuestra wawita

RESUMEN BIOGRÁFICO

Nací en Oruro, Bolivia el 3 de abril de 1978. A mis siete años mi familia se mudó a la ciudad de Cochabamba, donde realicé todos mis estudios formativos. A pesar del recelo de mis padres por mi elección de carrera profesional, ingresé a la carrera de Licenciatura en Biología en la Universidad Mayor de San Simón en 1996, carrera que no solamente me permitió desarrollar mi curiosidad científica, sino también me abrió las puertas a una gran parte de los rincones maravillosos de Bolivia. A media carrera (1999), ingresé como ayudante de investigación al incipiente Centro de Biodiversidad y Genética (CBG): me siento orgullosa de ser la primera generación de estudiante/tesista producido íntegramente en el CBG. Allí aprendí mucho sobre fragmentación de hábitats y ecología de aves, ya que mi tesis de licenciatura versó sobre la conducta territorial de un ave especialista de bosques de *Polylepis*. En 2002, a dos días de defender mi tesis de licenciatura me embarqué hacia Chile, a participar de una pasantía de investigación LANBIO en el laboratorio de quien es ahora mi tutor, Hermann M. Niemeyer. En esta ocasión, pude seguir profundizando mi incipiente conocimiento de ecología conductual y especialización ecológica usando como modelos dos variedades de *Myzus persicae*. Durante esta experiencia aprendí mucho acerca de la mediación de la química sobre las interacciones ecológicas y conocí el sistema de *Tristerix verticillatus*. Retorné a Bolivia el 2004, año que me dediqué a preparar currículo para postular al doctorado en Ecología y Biología Evolutiva de la Universidad de Chile, decidida a trabajar en interacciones ecológicas desde la perspectiva químico-ecológica. Durante estos años de doctorado, he seguido aprendiendo, he conocido científicos que han dejado profunda huella en mi pensamiento científico y en mi vida, he producido y me he reproducido. En el ámbito académico, puedo decir que el doctorado me ha permitido desarrollar mi pensamiento crítico y me ha enseñado sobre rigurosidad objetiva.

AGRADECIMIENTOS

Agradezco a Hermann M. Niemeyer, quien confió en mi persona desde 2002, y siempre estuvo ahí en cada paso de mi carrera profesional y mi vida adulta. Muchas gracias Hermann.

Deseo agradecer a mis tres tesistas: Andrea Lemaitre, Marioli Saldías y Francisca Lilienfeld, quienes han sido las manos que me han acompañado durante este viaje y cuyo entusiasmo y predisposición han sido indispensables para su buen término. También agradezco a todos mis amigos del Laboratorio de Química Ecológica, por la amistad, contribución y ayuda permanentes en las buenas y malas.

Agradezco a los miembros de mi comisión, Ramiro Bustamante, Sylvain Faugeron, Daniel Frías y Rodrigo Ramos, quienes presididos por Rodrigo Medel han contribuido sustancial y constructivamente a mi disertación doctoral, desde el planteamiento del proyecto hasta su culminación. He valorado mucho todos sus comentarios y opiniones.

Agradezco también a mis compañeros de doctorado, en especial al *círculo ecléctico de Ñuñoa* (Marce, Cata, Pablo, Karin y Ren) con quienes compartimos tantas noches de discusiones fabulosas de ciencia y quienes fueron los primeros en escuchar y contribuir con el planteamiento de la tesis.

Agradezco a mi maravillosa familia en Bolivia, quienes no dudaron en pegar el saltito a la cordillera cuando necesité la ayuda extra para finiquitar la tesis; muchas gracias por su apoyo incondicional.

Finalmente, agradezco a mi esposo, Renzo Vargas, quien ha sido mi cómplice y pilar fundamental y ha estado ahí cuando he necesitado, y por sobre todo me ha dado a Nataniel, nuestro tesoro. Te amo Ren.

FINANCIAMIENTO

Mis estudios de doctorado y la presente tesis han recibido el financiamiento de:

Beca de estudios de doctorado – Universidad de Chile a través del Proyecto MECESUP UCO-0214,

Gastos operativos: Proyecto IFS 4356-1 y Proyecto FONDECYT 1080248

Becas para tesistas: LANBIO y Proyecto FONDECYT 1080248

INDICE DE MATERIAS

DEDICATORIA ii

RESUMEN BIOGRÁFICO iii

AGRADECIMIENTOS vi

FINANCIAMIENTO v

RESUMEN xi

ABSTRACT xii

INTRODUCCIÓN 1

OBJETIVO GENERAL 4

OBJETIVOS ESPECÍFICOS 5

HIPÓTESIS 5

MÉTODOS 6

Capítulo 1: Preferencia de hospedero por un muérdago: infección desproporcional sobre tres hospederos coexistentes influenciada por el éxito de establecimiento diferencial

Abstract 10

Introduction 11

Methods 13

Results 19
Discussion 24
Acknowledgements 28
References 29

Capítulo 2: Efecto del hospedero sobre las interacciones entre un muérdago y sus polinizadores y herbívoros: un análisis de redes

Abstract 35
Introduction 36
Methods 38
Results 45
Discussion 56
Acknowledgements 59
References 59

Capítulo 3: Efectos ascendentes mediados por el hospedero sobre el movimiento de polen dentro de una población de muérdago

Abstract 66
Introduction 68
Methods 70
Results 78
Discussion 85

Acknowledgements 88

References 88

DISCUSIÓN GENERAL 93

REFERENCIAS 97

ÍNDICE DE TABLAS

Capítulo 1

Table 1. Performance of cross-inoculated seeds of *T. verticillatus* in relation to the origin and inoculation hosts, and passage through *M. thenca* digestive tract. Fisher exact tests were used to compare the two origins of seeds at each stage of development; significant *P* values are shown in bold....................24

Capítulo 2

Table 1. Types of herbivory found in *T. verticillatus* on the three host species....................42

Table 2. Diversity indexes for pollinators and herbivores of *T. verticillatus* on the three host species. Different letters in each diversity test indicate differences between groups after a permutation test ($P = 0.001$)........49

Table 3. Network-level parameters for the quantitative bipartite networks of *T. verticillatus* on three host species, and its pollinators and herbivores....................52

Capítulo 3

Table 1. Variables assessed for each hemiparasite-host system (mean ± SE). Comparisons were performed using Kruskal-Wallis ANOVA on ranks;

different letters denote significant differences between HHS at *P*<0.05 using a posteriori comparison tests.......79

ÍNDICE DE FIGURAS

Capítulo 1

Figure 1. Availability of hosts, measured as number of individuals/ha, and appearance of hosts, measured as height, area of projection to the ground and volume. Different letters in each graph denote significant differences at $P < 0.05$ between hosts 21

Capítulo 2

Figure 1. Rank-abundance curves representing the changes in heterogeneity in pollinator visits to each HHS. Pollinator taxa identified through capital letters as follows: *Sephanoides sephaniodes* (A), *Oretrochilus leucopleurus* (B), *Tatochila mercedis* (C), *Vanessa carye* (D), *Homoeonympha boisduvali* (E), *Episyrphis* sp. (F), *Tachinidae* sp. 1 (G), *Vespula germanica* (H), *Vespidae* sp. 2 (I), *Ichneumonidae* sp. 1 (J), *Bombus dahlbomi* (K), *Bombus terrestris* (L), *Apis mellifera* (M), *Megachile* sp. (N), Diptera sp. 1 (O), Diptera sp. 2 (P), Halictidae sp. 1 (Q), Halictidae sp. 2 (R), Lepidoptera sp. 1 (S), *Yramea cytheris* (T) 45

Figure 2. Rarefaction curves for the three HHS together (A) and species-accumulation curve for each HHS separately (B, C, and D) 48

Figure 3. Pollinator – HHS web. A) Network grid where the level of grey of each cell depicts the intensity of pair wise interaction dependence between each HHS and each pollinator species. B. Quantitative bipartite network. Codes for pollinators are shown in figure 1 53

Figure 4. Interaction norms showing host and abundance effects on the interaction strengths of mistletoe-herbivore (A) and mistletoe-pollinator (B) networks 54

Figure 5. Herbivore – HHS web. A) Network grid where the level of grey of each cell depicts the intensity of pair wise interaction dependence between each HHS and each herbivore type. B) Quantitative bipartite network. Codes for types of herbivory are shown in table 1 55

Capítulo 3

Figure 1. The mistletoe *Tristerix verticillatus*. (A) an intact inflorescence; (B) close-up of donor flower with fluorescent powder on anthers; (C) receiving flowers with fluorescent powder under white light; (D) the same receiving flowers in C under UV light. Note the different colors of fluorescent powder on the receiving flowers in D, indicating the arrival of powder from different donor HHS 74

Figure 2. Infrapopulations of T. verticillatus available at the study site (A), donor infrapopulations of each HHS designated for the release of fluorescent powder (B), infrapopulations with their respective floral display

size (C), and number of flowers of each HHS infrapopulation that received fluorescent powders (D). Each circle represent an infrapopulation and HHS are denoted by different colours: TS = red, TF = yellow, and TB = green. Data on number of flowers available (C) and number of receiving flowers (D) was log10 transformed to compensate for over dispersion of data; infrapopulations with no receiving flowers are shown by X's......75

Figure 3. Number of infrapopulations (a) and flowers (b) of different HHS that received fluorescent powders from different donor HHS. Median and interquartile ranges are shown and different letters within each HHS denote significant differences at *P*<0.05 using the a posteriori comparison tests..83

Figure 4. Pollen analogue arrival with respect to donor infrapopulations. A) Number of infrapopulations which received fluorescent powders as a function of the distance between donor and receiving infrapopulations, for different donor HHS, B) Moran's I correlogram depicting the absence of spatial autocorrelation on pollen reception..84

RESUMEN

Los compuestos volátiles emitidos por y los metabolitos secundarios contenidos en individuos de una población del muérdago *Tristerix verticillatus* (Loranthaceae) varían en función a la especie de hospedero que parasita en un área donde infecta tres especies de hospedero simultáneamente (*Schinus montanus, Fabiana imbricata* y *Berberis montana*). Las diferencias en los compuestos volátiles afectan las visitas florales por parte de insectos, pero no sobre las visitas florales por aves; en cuanto a los metabolitos secundarios, se ha registrado la traslocacion de alcaloides desde *B. montana* hacia *T. verticillatus*. Este conjunto de evidencias permiten hipotetizar que las especies de hospedero ejercen una influencia ascendente sobre las interacciones mutualistas y antagonistas de *T. verticillatus.* Así, se predice que: i) los hospederos ejercen una influencia sobre las interacciones que mantiene *T. verticillatus* con sus polinizadores y herbívoros, determinando así diferencias tanto en la diversidad de especies así como en sus respectivas fuerzas de interacción, y ii) la influencia que ejercen los hospederos al nivel de polinizadores también incide sobre el intercambio de polen (flujo génico potencial) dentro la población de *T. verticillatus.* Se comprobaron estas predicciones mediante dos estudios complementarios entre sí: i) la determinación de la influencia del hospedero sobre la diversidad de polinizadores y herbívoros, así como sobre la estructura de sus redes de interacción, y ii) la determinación del movimiento de polen potencial entre sistemas parásito-hospedero. De esta manera se determinó el efecto de hospederos co-ocurrentes sobre la estructuración de la comunidad de herbívoros y polinizadores, y su alcance como potencial mecanismo de diferenciación genética.

ABSTRACT

The secondary chemistry of internal metabolites, as well as the volatile chemistry of the mistletoe *Tristerix verticillatus* (Loranthaceae) differ between mistletoe individuals that parasitise three distinct co-occurring host species (*Schinus montanus*, *Fabiana imbricata* and *Berberis montana*). The differences in the volatile chemistry have shown to affect flower visits by insects but not by birds; moreover, the traslocation of isoquinolinic alkaloids has been recorded for *T. verticillatus* parasitising *B. montana* only. Thus, this set of evidences allow to hypothesise that host species exert a bottom-up influence on the mutualistic and antagonistic interactions of *T. verticillatus*. Hence, the following predictions are made: i) host species will determine differences in the species diversity as well as in the interaction strength of the pollinator and herbivore community of interacting species of *T. verticillatus*, and ii) the influence of host on the interaction of the mistletoe with its pollinators will also determine the pattern of pollen movement (potential gene exchange) within the mistletoe population. The issues raised were assessed through two complementary studies: i) host effects on the interactions between the mistletoe and its pollinators and herbivores through a network analysis, and ii) host effects on pollen movement within the mistletoe population. Thus, the effect of host on the structuring of the herbivore and pollinator community, and its importance as potential mechanism of genetic differentiation were determined.

INTRODUCCIÓN

"The parallel in ecological and evolutionary pattern between parasitic seed plants and herbivorous insects is so striking that knowledge from one should generate similar predictions about the other" (Atsatt, 1977)

Las interacciones que mantienen los insectos fitófagos con sus plantas hospederas pueden considerarse análogas a las interacciones que mantienen las plantas parásitas con sus plantas hospederas (Pennings & Callaway 1991, Atsatt 1977). De hecho, plantas parásitas e insectos fitófagos –en conjunto con viruses, parasitoides, endo- y ectoparásitos de animales tales como flagelados, artrópodos y nemátodos– están incluidos dentro del concepto de "parásito" (Poulin 2007), que se define como aquel organismo que vive en o sobre otro organismo –el hospedero del cual se alimenta– mostrando algún grado de adaptación estructural a él y causándole algún daño (Huyse et al. 2005).

En insectos fitófagos, diferencias en el patrón de uso de hospederos pueden a su vez resultar en diferenciación fenotípica (a nivel conductual, morfológico y/o fisiológico), la cual a su vez puede incidir en su reproducción al generar mecanismos de apareamiento estructurado (*assortative mating*: apareamiento preferencial entre fenotipos similares, i.e. Caillaud & Via 2000) que en última instancia puede llevar a la formación de razas especializadas y eventualmente a la especiación en simpatría (Berlocher & Feder 2002).

En plantas fanerógamas, la reproducción sexual depende de la deposición de polen en el estigma por medio de agentes de polinización (bióticos y abióticos). Por esto, es necesario considerar a la planta hospedero como mediadora en las relaciones que mantienen las plantas parásitas con sus polinizadores, para así determinar el rol de las plantas hospederas sobre la ocurrencia de diferenciación intrapoblacional que puede eventualmente conducir al origen de razas dentro una población de plantas parásitas, como ocurre en insectos fitófagos. No obstante, a pesar del evidente vínculo, sólo en contados estudios se ha examinado en profundidad la hipótesis de adaptación local y existencia de razas genéticas especializadas a sus hospederos en plantas parásitas (Norton & Carpenter 1998; Poulin & Mouillot 2003, Jerome & Ford 2002).

La mediación de los hospederos sobre las interacciones del parásito con otros organismos puede enmarcarse dentro del estudio de efectos ascendentes o *bottom up* (Hunter & Price 1992, Power 1992, Roughgarden et al. 1994). A la fecha, algunos estudios han demostrado la ocurrencia de efectos ascendentes que escalan a través de sistemas planta hospedero – planta parásita – mutualistas y/o antagonistas (i.e. Lei 1999, Medel et al. 2004, ván Ommeren & Whitham 2002). En cuanto a la mediación del hospedero sobre la divergencia fenotípica en plantas parásitas con incidencia directa sobre los niveles tróficos superiores, es de particular interés el caso de las hemiparásitas de raíz del género *Castilleja* que pueden parasitar especies de hospedero que difieren en su contenido de alcaloides, los cuales a su vez son traslocados a la planta hemiparásita; se ha observado que la herbivoría, la polinización y la reproducción de *C. indivisa* difieren en relación al contenido de alcaloides en ella, el que a su vez es determinado por el hospedero parasitado (Adler 2000; Adler 2003).

Así también, las plantas hospederas pueden incidir sobre las características del fenotipo en compuestos químicos volátiles de sus plantas parásitas; tal es el caso del muérdago *T. verticillatus* (Loranthaceae) cuando parasita los hospederos *Schinus montanus* (Anacardiaceae), *Fabiana imbricata* (Solanaceae) y *Berberis montana* (Berberidaceae) en simpatría. Esta especie de muérdago presenta un polimorfismo químico tanto cualitativo como cuantitativo, el que es mediado por las especies de hospedero que utiliza. Este polimorfismo químico a su vez, tiene una directa incidencia sobre el número total de visitas por polinizadores, así como sobre la tasa de visitas de polinizadores específicos (i.e. Apidae), lo que se ha atribuido principalmente a que los morfotipos químicos poseen de manera diferencial compuestos que se han comprobado como atrayentes o repelentes hacia algunos grupos de insectos (Troncoso et al. 2010). También se ha evidenciado la traslocación de alcaloides isoquinolínicos hacia *T. verticillatus* desde *B. montana*, fenómeno que no ocurre cuando el hemiparásito infecta los otros dos hospederos (Cabezas et al. 2009).

Dada la analogía que puede trazarse entre la relación trófica que mantienen los insectos herbívoros con sus plantas hospederas y las plantas parásitas con sus plantas hospederas, Atsatt (1977) propuso que a partir del amplio conocimiento que existe sobre la ecología de las interacciones herbívoros-hospederos se pueden generar predicciones comprobables para las interacciones parásito-hospedero en plantas.

La presente tesis propone que, así como ocurre en insectos fitófagos, como producto de la diversificación fenotípica canalizada por el hospedero y su incidencia sobre la interacción con sus organismos interactuantes, éstos pueden conducir a generar reproducción estructurada dentro la población de *T. verticillatus*. Por tanto, tomando en consideración los efectos ascendentes que las tres especies de hospedero ejercen sobre los compuestos volátiles emitidos por *T. verticillatus* y sobre las tasas de visita por parte de sus polinizadores, surgen las siguientes preguntas:

i) ¿Cómo varía la influencia ascendente de las tres especies de hospedero sobre las interacciones de polinización (mutualista) de *T. verticillatus*?

ii) ¿Cómo varía el movimiento de gametos (análogos de polen) dentro la población de *T. verticillatus*, como resultado de la influencia de las especies de hospedero sobre sus interacciones con polinizadores?

OBJETIVO GENERAL

— Determinar la influencia del hospedero sobre las interacciones de *T. verticillatus* con sus polinizadores y sus efectos sobre el flujo de polen entre individuos parásitos sobre distintos hospederos.

OBJETIVOS ESPECÍFICOS

- Comparar la influencia que ejercen las tres especies de hospedero sobre las interacciones que mantiene *T. verticillatus* con sus polinizadores a través de una comparación de la diversidad de especies que interactúan con *T. verticillatus* así como de sus respectivas fuerzas de interacción, a través de un análisis de redes de interacción
- Comparar el flujo de polen dentro de la población de *Tristerix verticillatus* que parasita tres especies de hospedero que coocurren dentro un área determinada

HIPÓTESIS

Debido a la canalización fenotípica que ocurre como consecuencia del parasitismo sobre distintos hospederos se predice que las especies hospederas ejercen una influencia ascendente sobre la intensidad de interacción entre la planta parásita y sus polinizadores, lo cual tiene un efecto directo sobre su producción de frutos

Debido a la influencia ascendente que ejercen las especies hospederas sobre la intensidad de interacción de la planta parásita con sus polinizadores, se predice que se pueden detectar diferencias en el patrón de movimiento de polen dentro la población de *T. verticillatus.*

METODOS

El género *Tristerix* (Santalales: Loranthaceae) está restringido al continente sudamericano, en la cordillera andina desde Colombia hasta el sur de Chile, donde también ocurre en la cordillera de la costa (Kuijt 1988). La especie *Tristerix verticillatus*, también denominado "quintral del molle", se distribuye desde el sudeste de Bolivia y noroeste de Argentina hasta la zona central de Mendoza, y en Chile, desde el sur de Atacama hasta el sur de Valdivia en hábitats boscosos desde el nivel del mar hasta los 2500 m.s.n.m. aproximadamente (Kuijt 1988, información local brindada por el Herbario del MNHN, 2006). Dentro del género *Tristerix* existe amplia variación en su rango de hospederos y también en su especificidad de hospedero (*sensu* Lymbery 1989). *Tristerix verticillatus* parasita 22 especies de hospederos, la mayor parte de los cuales pertenece a órdenes distintos (STD=3.83, V(STD)=0.39) (índices de especificidad desarrollados por Poulin & Mouillot 2003), aunque el género *Schinus* ha sido descrito como su hospedero principal sobre la base de la frecuencia de colectas botánicas reportadas (Kuijt 1988) y pese a no existir evidencias ecológicas que demuestren adaptación diferencial en comparación a otros hospederos. No obstante, muchos parásitos descritos como generalistas en la literatura han demostrado en una inspección más detallada ser especialistas a nivel local, es decir que aunque son capaces de usar un gran número de hospederos, son especialistas de sólo algunos hospederos en un área determinada (Norton & Carpenter 1998).

Área de estudio

Los estudios que conforman esta tesis fueron realizados dentro del Santuario de la Naturaleza Yerba Loca (33° 18' S, 70° 19' O, 1970 m.s.n.m.), ubicado en la Comuna de Lo Barnechea, a 70 Km en dirección noreste de Santiago, Chile. Dentro de este santuario existe una población de *T. verticillatus* que parasita tres especies de hospederos que ocurren en simpatría: *Schinus montanus* (Sapindales, Anacardiaceae), *Fabiana imbricata* (Solanales, Solanaceae) y *Berberis montana* (Ranunculales, Berberidaceae). Cada sistema hemiparásito-hospedero (HHS por sus siglas en inglés) fue designado como: TS (*T. verticillatus* infectando *S. montanus*), TF (*T. verticillatus* infectando *F. imbricata*) y TB (*T. verticillatus* infectando *B. montana*), nomenclatura a la que se hará referencia de aquí en adelante. Asimismo, utilizo el término infrapoblación, para referirme a todos los individuos hemiparásitos presentes en un solo individuo hospedero (Huyse et al. 2005).

Para responder las preguntas y comprobar las predicciones enunciadas, se han planteado tres estudios que se desarrollan en tres capítulos, los cuales describo brevemente a continuación.

- *Capítulo 1: Preferencia de hospedero por un muérdago: infección desproporcional sobre tres hospederos coexistentes influenciada por el éxito de establecimiento diferencial.* Este estudio ha sido planteado para determinar los patrones de infección de *T. verticillatus* (incidencia e intensidad de infección) sobre las tres especies de hospedero, y además evaluar si los patrones de infección observados se relacionan con diferencias durante el proceso de infección (desde la germinación hasta el establecimiento) que se observa en experimentos de inoculación artificial. A través de este estudio, se logra además recopilar información con respecto a la biomasa vegetal de *T. verticillatus* en relación al hospedero infectado.

- *Capítulo 2: Efectos ascendentes del hospedero sobre las interacciones entre un muérdago y sus polinizadores y herbívoros.* En este estudio se somete a prueba la primera hipótesis planteada, analizando las interacciones del hemiparásito con sus polinizadores; se incluye además al ensamble de herbívoros, dada su potencial importancia para evaluar objetivamente la asociación que existe entre la polinización y la producción final de frutos en *T. verticillatus* para cada sistema hemiparásito-hospedero (criterio de acuerdo a Strauss 1997).
- *Capítulo 3: Efectos ascendentes mediados por el hospedero sobre el movimiento de polen dentro de una población de muérdago.* En este estudio se somete a prueba la segunda hipótesis planteada, a través de una aproximación experimental mediante el uso de polvos fluorescentes como análogos de polen, que permiten detectar el patrón de movimiento del polen dentro de la población de *T. verticillatus* para cada sistema hemiparásito-hospedero.

CAPÍTULO 1

Preferencia de hospedero por un muérdago: infección desproporcional sobre tres hospederos coexistentes influenciada por el éxito de establecimiento diferencial

(Host preference of a temperate mistletoe: disproportional infection on three co-occurring host species influenced by differences in establishment success)

Abstract The mistletoe *Tristerix verticillatus* (Loranthaceae) parasitises within a small area of the Yerba Loca Nature Sanctuary near Santiago, Chile, three co-occurring hosts: *Schinus montanus* (Anacardiaceae), *Fabiana imbricata* (Solanaceae) and *Berberis montana* (Berberidaceae). Previous studies suggest that *T. verticillatus* may be favoured when parasitising *S. montanus* relative to the other two host species. We hypothesize that infection of *S. montanus* is not proportional to its local abundance or appearance, that *S. montanus* is more intensively parasitised than other available hosts, and that host provenance is a determinant of the fate of the infecting seed. We compare the incidence of infection of *T. verticillatus* in relation to local availability and appearance variables, and the intensity of infection of *T. verticillatus*, on the three co-occurring host species. We then test the effects of host provenance on mistletoe seed establishment success with a seed cross inoculation experiment varying the donor and receptor hosts. Finally, we test whether there are differences in establishment success between manually processed seeds and seeds defecated by the avian disperser *Mimus thenca* (Passeriformes: Mimidae). Our results show that the three hosts have an aggregated spatial distribution. *Schinus montanus* was parasitised at a higher rate than expected by its local availability and appearance, and inoculated seeds showed differential development depending on the origin of the seeds: seeds from *T. verticillatus* parasitising *S. montanus* inoculated to *S. montanus* twigs showed higher germination and lower mortality than seeds from *T. verticillatus* parasitising *F. imbricata* inoculated to S. montanus twigs. Furthermore, seeds defecated by the avian disperser, *Mimus thenca*, had higher adherence and reduced mortality when compared to manually processed seeds. The disproportional host infection found is discussed in terms of the differential establishment of mistletoe seeds, morphological characteristics of hosts and the behaviour of dispersing birds.

Key words: disproportional infection, host-parasite interaction, host preference, Loranthaceae, seed cross inoculation

INTRODUCTION

Parasitic plants are considered similar to herbivores in relation to interactions with their hosts (Attsat 1977; Pennings & Callaway 2002). Like herbivores, parasitic plants can preferentially infect hosts that provide greater return in nutrition, reproduction and/or survival (Pennings & Callaway 2002; Press & Phoenix 2005). Host preference by a parasitic plant refers to the disproportional infection in relation to the availability of hosts in the environment; this may result from an active selection of the host by the parasite (Runyon et al. 2006), from ecological factors such as preferential attraction of pollinators or dispersers (Troncoso et al. 2010), or from host-parasite compatibility mechanisms (Press & Phoenix 2005).

Mistletoes are parasitic plants of the Loranthaceae and Viscaceae that are considered key components of ecosystems on account of the variety of interactions they establish with other organisms (Watson 2001; Press & Phoenix 2005). Mistletoes vary widely in their degree of host specificity (Press & Phoenix 2005). This variation can be found even within a single genus, such as *Tristerix* (Loranthaceae). Thus, while *T. aphyllus* and *T. chodatianus* have been only found infecting species of Cactaceae and Rosaceae, respectively (in fact, *T. chodatianus* has only been collected on plants of the genus *Polylepis*), *T. corymbosus* has been recorded infecting about 30 host species of 21 different plant families (Kuijt 1988; Troncoso unpubl.). Nonetheless, generalist parasites are not fully unrestricted in their host range; on the contrary, they may show local preference for some hosts over others, and such pattern of host use has been related to the local abundance of hosts (Norton & Carpenter 1998). In general, the pattern of mistletoe infection within a given area depends primarily on three factors: i) the spatial distribution of hosts, which affects the likelihood of encounter with mistletoe dispersers (Norton & Carpenter 1998; Medel et al. 2002); ii) vector (i.e. birds) occurrence and behaviour, which affects the probability of infection (Aukema & Martínez del Río 2002a; Aukema 2004; Medel et al. 2004; Roxburgh & Nicolson 2005); and iii) the differential development of mistletoe seeds, which is generally higher on the host species of provenance (Clay et al. 1985, Rödl & Ward 2002) and hosts with higher resource availability (Watson 2009).

The mistletoe *Tristerix verticillatus* R. et P. (Loranthaceae) is a shrubby hemiparasite which is distributed east of the Andes from southeastern Bolivia (Santa Cruz) to northwestern Argentina (up to Mendoza), and west of the Andes in northern and central Chile (from La Serena to Valdivia) and from sea level up to 2500 m.a.s.l (Kuijt 1988). Our study population occurs within the Yerba Loca Nature Sanctuary (YLNS), near Santiago (Metropolitan Region, Chile), where *T. verticillatus* parasitises three co-occurring shrubby species: *Schinus montanus* Phil. (Sapindales: Anacardiaceae), *Fabiana imbricata* R. et P. (Solanales: Solanaceae) and *Berberis montana* Gay (Ranunculales: Berberidaceae). Previous studies have shown that *T. verticillatus* produces more nectar, receives more floral visits and has reduced herbivory levels when it parasitises *S. montanus* compared to *F. imbricata* and *B. montana* (Troncoso et al. 2010;

Cabezas 2007), suggesting that *T. verticillatus* may be favoured when parasitising *S. montanus* relative to the other co-occurring host species (*F. imbricata* and *B. montana*). In the present study, we address the question of whether there is a preferential infection of *T. verticillatus* towards *S. montanus* when the other two hosts are available and enquire about possible proximate causes of the phenomenon. We hypothesize that infection of *S. montanus* is not proportional to its local abundance, that *S. montanus* is more intensively parasitised than other available hosts, and that host provenance is a determinant of the fate of the infecting seed. We test the hypothesis by comparing the incidence of infection of *T. verticillatus* in relation to local availability and the intensity of infection of *T. verticillatus*, on the three co-occurring host species; having found a disproportional infection pattern in the study area, we test the effects of host provenance on mistletoe seed establishment success with a seed cross inoculation experiment varying the donor and receptor hosts. In addition, we test whether there are differences in establishment success between manually processed seeds and seeds defecated by the avian disperser *Mimus thenca* (Passeriformes: Mimidae).

METHODS

Study area

The YLNS is located 25 km northeast of the city of Santiago (33° 10'-33° 22' S; 70° 13'-70° 24' W), between 900 and 5500 m.a.s.l. The study was conducted within the area called Villa Paulina (1900-2100 m.a.s.l.) situated on the west facing slope along the Yerba Loca creek. The plant community in this area is composed of graminaceous and cushion plants and small bushes with hard and perennial leaves (Gajardo 1994). Among these latter species, three hemiparasite-host systems co-occur (*T. verticillatus* parasitising *S. montanus*, *F. imbricata* and *B. montana*, respectively). *Fabiana imbricata* grows on the drier slopes of the study area, *B. montana* is found by the Yerba Loca creek and nearby meadows, and *S. montanus* is predominantly found along the Yerba Loca creek. Other *Tristerix* species occurring in the YLNS (*T. corymbosus* and *T. aphyllus*) parasitise other hosts and do not occur in the study area.

Host availability and appearance

To determine the availability of hosts and the incidence and intensity of infection of *T. verticillatus*, the total study area was divided into a grid of 180 plots of 50 x 50 m. Each plot was numbered. Random numbers were used to designate 20 evaluation plots; however, if adjacent plots occurred (thus over-emphasizing a given type of habitat), another set of random numbers was used until no adjacent plots occurred. Evaluation plots covered about 11% of the total study area. The availability and appearance of hosts in the evaluation plots were assessed through the estimation of their relative abundance, and their individual height, area and volume, respectively. Relative abundance was measured as the number of individuals of each host species in each evaluation plot. The height, area and volume were recorded for all host individuals within the evaluation plots. Because the three host species closely resemble hemiellipsoids, the area of their projection to the ground surface was calculated with the formula of an ellipse and the volume with the formula of a hemiellipsoid using the projected area and height of the individual. Host abundance was compared with one-way Kruskal-Wallis tests followed by post-hoc Tukey tests (Sheskin 2000) and host area, height and volume were compared with one-way Kruskal-Wallis tests followed by post-hoc Dunn's tests due to unbalanced data sets (Siegel & Castellan 1988).

Host spatial distribution

Thirty geographical coordinates (points) were randomly located within the study area and from each point the T-square method (Besag & Gleaves 1973, Krebs 1999) was used to measure the distances between two close neighbour host individuals. In this method, random points are located and at each of them two distances are measured: the distance from the random point to the nearest organism and the distance from the organism to its nearest neighbour, with the restriction that the angle between the random point, the nearest organism and its nearest neighbour must be more than 90°. The pattern of spatial distribution was obtained by calculating Hines test statistic, ht, and the level of statistical significance was compared with values obtained with the Fisher distribution. The rejection levels of the null hypothesis for random distribution were calculated for 60 degrees of freedom. A random pattern was inferred if $0.60 < ht < 1.66$, a uniform pattern if $ht < 0.60$, and an aggregated pattern if $ht > 1.66$ (Krebs 1999). This method was used to assess the spatial distribution of all host individuals, jointly and each host species separately, and all hemiparasite infrapopulations (i.e., all individuals of the hemiparasite occurring on a given host individual; Huyse 2005) in the study area. In the case of the hemiparasite, the method was carried out for the totality of hemiparasite infrapopulations regardless of the host species parasitised, in order to obtain their overall spatial distribution; subsequently, the spatial distributions of the mistletoe parasitising each of the three host species were re-evaluated separately to compare the distribution pattern in relation to the parasitised host species.

Incidence and intensity of infection

The incidence of infection was separately evaluated for each host species as the ratio between the number of all parasitised host individuals and the total number of available host individuals within the evaluation plots (Kelly et al. 1988; Norton & De Lange 1999; Dzerefos et al. 2003). In order to compare host use with host availability, Manly standardised selection indices (Bi) (Krebs 1999) were used. These indices indicate a host preference when the proportion of infected hosts is higher than expected from their local availability. The values of the selection indices were compared with the expected ratios for non-preference (0.33) using the G test for partial samples of the total population (Krebs 1999).

The intensity of infection was determined in two ways: i) as the ratio between the sum of the volumes of all parasitic plants per host individual and the volume of that host individual, and ii) as the number of hemiparasite individuals per host individual. The intensities of infection were compared between hosts with the Kruskal-Wallis test. A posteriori comparisons were made using the non-parametric Dunn test for comparisons of unbalanced designs (Siegel & Castellan 1988).

Germination, adherence, establishment and mortality

A cross inoculation experiment (Kawecki & Ebert 2004) was set up to study the effect of provenance and destination host species on the development of *T. verticillatus* seeds. One hundred and twenty fruits (ripe and without herbivore damage) were collected from "donor" hosts (provenance effect): 60 of *T. verticillatus* parasitising *S. montanus* and 60 of *T. verticillatus* parasitising *F. imbricata*; no fruits were collected from *T. verticillatus* parasitising B. montana because this system did not produce enough fruits for the experiments planned. The fruits collected were stored in a cooler until planted the day after (Sargent 1995; Norton et al. 2002). Before placing the fruits on the receptor hosts (destination effect), the exocarp was removed from them by carefully squeezing the fruits to expose the seed's viscin (Ladley et al. 1997; Rödl & Ward 2002). In

60 uninfected receptor host individuals (30 replicates per host species), 7-20 mm-thick branches with similar orientation and at similar height were chosen and two peeled fruits were placed on each of them, one from *S. montanus* and one from *F. imbricata*. At the time of planting, twigs with inoculated seeds were swayed to verify if seeds were well stuck and then they were sprinkled with water to closely simulate the humidity present in a recent bird dropping. Seeds were checked after 5, 10, 20, 40 and 60 days of planting, to record if the haustorium had emerged and had began its growth in length (germination), the haustorial disk had formed and attached to the twig (adherence), the leaf primordia had appeared (establishment), or they had died (Powell & Norton 1994; Norton et al. 2002). Natural death of seeds was observed as a progressive dehydration, shrinkage and turning black, which eventually led to the seed falling off from the inoculated twig. Overall mortality encompassed seeds that died naturally, seeds that were predated and seeds that fell to the ground while alive. The number of seeds that accomplished or not a given stage of development and the number of dead seeds were counted, and these numbers were compared between different provenances (*T. verticillatus* from *S. montanus* and *F. imbricata*) using a one-tailed Fisher's exact test (Sheskin 2000).

Another experiment was set up to compare the performance of fruits whose exocarp had been manually removed with fruits that had passed through the digestive system of the disperser bird, *M. thenca*. Sixty ripe and undamaged fruits of *T. verticillatus* growing on *S. montanus* were collected. One set of 30 fruits were fed to a caged specimen of *M. thenca* caught at the study site and subjected previously to one day of fasting. Seeds were recovered from the bird's faeces. The exocarp of the other set of 30 fruits was removed manually as described above. Seeds (defecated and manually processed) were then placed pairwise on branches of infected *S. montanus* and observed as described above. Seeds were counted and their numbers analysed as described in the preceeding paragraph.

RESULTS

Host availability and appearance

The three host species differed significantly in their relative abundance (H = 26.918, P < 0.001), area (H = 10.75, P = 0.005), volume (H = 8.87, P = 0.01) but not in height (H = 3.64, P = 0.16). *Fabiana imbricata* was the most abundant host with 75.9 ± 15.1 (mean ± SE) individuals per hectare on average followed by *B. montana* (18.8 ± 6.8 individuals/ha) and *S. montanus* (5.7 ± 2.2 individuals/ha). Moreover, *F. imbricata* was the most voluminous host with 44.29 ± 25.19 m3 on average followed by *B. montana* (8.45 ± 0.47 m3) and *S. montanus* (6.08 ± 1.28 m3), and *F. imbricata* was also the host with the greatest area with 1.24 ± 0.20 m2, followed by *B. montana* (1.90 ± 0.04 m2) and *S. montanus* (1.24 ± 0.20 m2) (Fig. 1).

Spatial distribution

All hosts considered as one group independent of the species and also each host species separately, presented an aggregated spatial distribution, ht being greater than 1.66 in all cases (All hosts = 3.58, *S. montanus* = 2.95, *F. imbricata* = 3.44, *B. montana* = 3.81). Similarly, the total mistletoe population (all infrapopulations on the three host species) presented an aggregated spatial distribution (ht value for *T. verticillatus* = 1.90); furthermore, mistletoe infrapopulations also showed aggregated spatial distribution when analysed independently for each host species (ht values for *T. verticillatus* on *S. montanus* = 2.07, *T. verticillatus* on *F. imbricata* = 1.92, *T. verticillatus* on *B. montana* = 2.74).

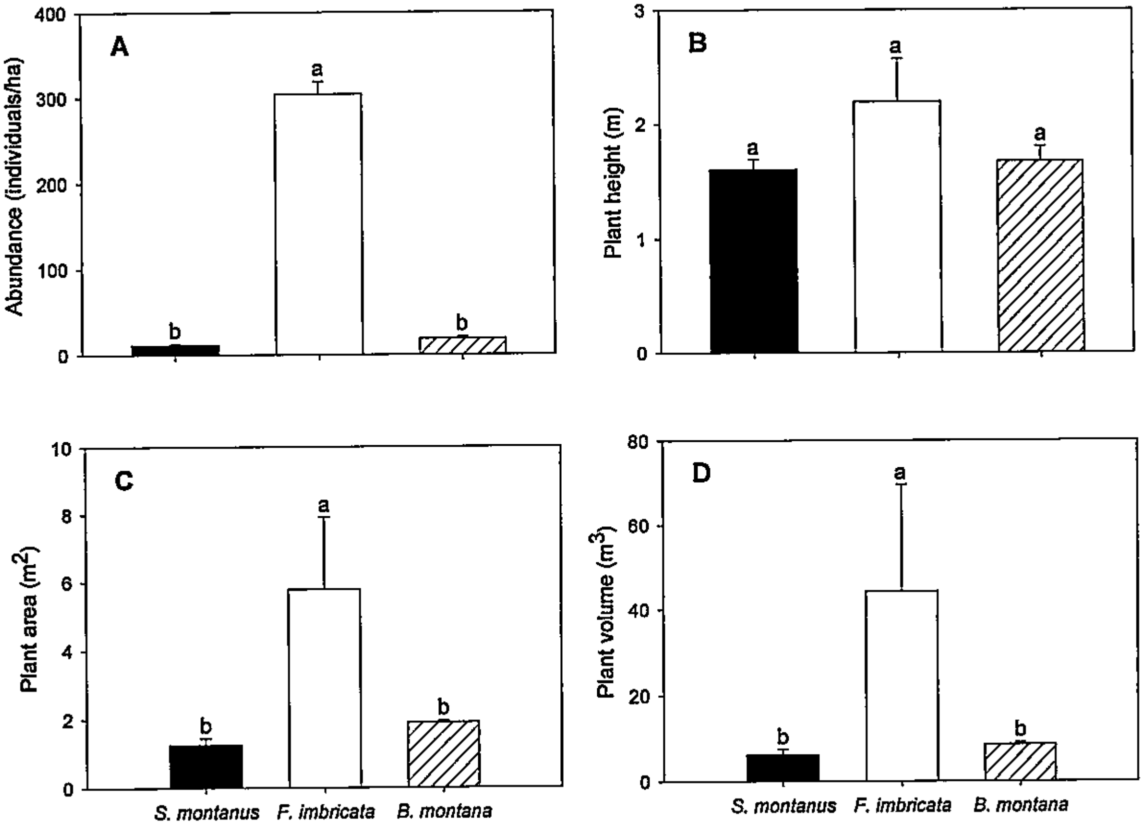

Figure 1. Availability of hosts, measured as number of individuals/ha, and appearance of hosts, measured as height, area of projection to the ground and volume. Different letters in each graph denote significant differences at $P < 0.05$ between hosts.

Incidence and intensity of infection

T. verticillatus did not parasitise the three available hosts to the same extent (G test: $X^2 = 17256.93$, $P < 0.05$). While *S. montanus* was parasitised in a proportion higher than that expected by its abundance (Bi = 0.971), *B. montana* and *F. imbricata* were parasitised in a lower proportion than that expected on the basis of their abundance (Bi = 0.029 and 0.0003, respectively).

The intensity of infection was measured in terms of number of hemiparasites per host individual and in terms of hemiparasite biomass (ratio of hemiparasite/host volumes). The mean number of hemiparasite individuals per host individual did not differ significantly between the three host species (7.8 ± 2.5, 13.3 ± 10.4, and 4.0 ± 3.0 for *S. montanus*, *F. imbricata* and *B. montana*, respectively; $H = 0.176$, $P = 0.916$). Nonetheless, in terms of biomass, the most intensively infected host was *S. montanus* ($H = 11.9$, $P < 0.005$) on which the hemiparasite occupied almost 10 % of the total host volume (10.4 ± 0.03 %) whereas it occupied less than 1% of the host volume when infecting *F. imbricata* and *B. montana* (0.4 ± 0.3 and 0.3 ± 0.2 %, respectively). Moreover, neither the mean number of hemiparasite individuals per host individual nor the hemiparasite/host volume ratio were correlated with any of the variables of host appearance, i.e. plant height ($r = 0.18$, $P = 0.31$; $r = -0.15$, $P = 0.42$, respectively), area ($r = 0.19$, $P = 0.28$; $r = -0.29$, $P = 0.10$, respectively), and volume ($r = 0.19$, $P = 0.28$; $r = -0.28$, $P = 0.12$, respectively).

Germination, adherence, establishment and mortality

Of the total of 120 seeds planted at the beginning of the cross inoculation experiment, five seeds (2.8%) were lost during the study (fell off or suffered predation), 69 (57.5 %) germinated, 21 (out of 69; 30.4 %) adhered, and only 7 (out of 21; 33.33 %) successfully established on the host twig. At the end of the study, the mortality of seeds of *T. verticillatus* was 94.2 % (113 seeds).

Seeds from *S. montanus* inoculated on *S. montanus* showed higher germination and reduced mortality than seeds from *F. imbricata* inoculated on *S. montanus* (Table 1). In the inoculation of *F. imbricata* with seeds of *T. verticillatus* from *S. montanus* and *F. imbricata*, the number of seeds did not differ in germination, adherence, establishment or mortality (Table 1). Furthermore, seeds defecated by *M. thenca* showed higher adherence than manually processed seeds and mortality was highest for manually processed seeds (Table 1).

Table 1. Performance of cross-inoculated seeds of *T. verticillatus* in relation to the origin and inoculation hosts, and passage through *M. thenca* digestive tract. Fisher exact tests were used to compare the two origins of seeds at each stage of development; significant *P* values are shown in bold.

Inoculated host	Origin of *T. verticillatus* seeds	Germination		Adherence		Establishment		Overall mortality	
		Yes	No	Yes	No	Yes	No	Died	Survived
S. montanus	*F. imbricata*	12	18	2	10	0	2	30	0
	S. montanus	24	6	11	13	5	6	25	5
		***P* = 0.002**		*P* = 0.09		*P* = 0.35		***P* = 0.03**	
F. imbricata	*F. imbricata*	18	12	4	14	2	2	28	2
	S. montanus	15	15	4	11	0	4	30	0
		P = 0.30		*P* = 0.65		*P* = 0.25		*P* = 0.25	
S. montanus	*S. montanus*, defecated	21	9	11	10	5	6	25	5
	S. montanus, peeled	25	5	1	24	0	1	30	0
		P = 0.18		***P* = 0.002**		*P* = 0.58		***P* = 0.03**	

DISCUSSION

Our results show that *T. verticillatus* infected *S. montanus* with greatest incidence and intensity (hemiparasite/host biomass ratio); however, the most abundant and apparent host (in terms of volume and area) was *F. imbricata*. Hence, infection was disproportional in relation to the availability of hosts in the study site; moreover, it was not correlated with host appearance. The disproportional use of hosts in relation to their availability has previously been observed in parasitic plants belonging to different plant families, e.g., Loranthaceae (Dzerefos et al. 2003), Convolvulaceae (Kelly et al. 1988), Orobancheaceae (Marvier & Smith 1997) and Santalaceae (Aukema & Martínez del Río 2002a), and has been attributed to three main mechanisms: the spatial distribution of hosts (Medel et al. 2002), the dispersing behaviour of birds (Aukema & Martínez del Río 2002b; Roxburgh & Nicolson 2005) and the differential establishment of mistletoe seeds (Aukema & Martínez del Río 2002a).

The pattern of host spatial distribution affects the probability of host contact with the mistletoe (Norton & Carpenter 1998, Medel et al. 2002). We have found that all hosts taken together as well as each of the three hosts taken separately have an aggregated spatial distribution; hence, it would be expected that after arriving on a host plant, mistletoes would spread to neighbouring host plants at rates independent of host species. Therefore, spatial aggregation cannot be considered an important factor leading to the disproportional infection of hosts.

The morphological characteristics and growth habitat of hosts have been shown to affect their infection by mistletoes (Watson 2009). *Fabiana imbricata*, in spite of being the most apparent and abundant host, grows on the drier slopes of the study area; hence, it may represent a non-preferred host causing water stress to the mistletoe (host quality hypothesis: Watson 2009). On the other hand, *B. montana* is found by the Yerba Loca creek and nearby meadows, which would seem advantageous in terms of avoiding water stress to the mistletoe while attracting the avian vector of the mistletoe, thus enhancing the probability of the vector spreading; however its spiny branches might deter the avian vector and thus reduce its chance of infection (Sargent 1995; Norton & Ladley 1998). Finally, *S. montanus* lacks spines and is predominantly found along the Yerba Loca creek. Overall, these data are not inconsistent with the prevalent infection of S. montanus relative to the other two co-occurring hosts.

Another factor which has been shown to affect mistletoe prevalence is the differential development success of seeds on different host species; hence a seed cross inoculation experiment was performed. The overall success of the inoculation experiment did not differ substantially from those reported in comparable experiments. Thus, germination (57.5 %) was not far from the range reported for other mistletoes (between 69 and 98 %: Clay et al. 1985; Ladley & Kelly 1996; Norton & Ladley 1998; Rödl & Ward 2002; Norton et al. 2002; Roxburgh & Nicolson 2005; all reports on Loranthaceae mistletoes) while adherence (30.4 % - values reported between 6 and 26 %: Clay et al. 1985; Rödl & Ward 2002), establishment (33.3 % - values reported between 2 and 48 %: Clay et al. 1985; Ladley & Kelly 1996; Norton et al. 2002) and mortality (94.2 % - values reported between 71 and 97 %: Clay et al. 1985; Ladley & Kelly 1996; Roxburgh & Nicolson 2005) were within the range reported for other mistletoes.

Germination of seeds in the mistletoe is generally high and occurs under almost all circumstances (Lamont 1983; Kuijt 1969, 1988; Norton & Carpenter 1998). However, seeds of *T. verticillatus* from *S. montanus* showed higher germination than seeds from *F. imbricata* when inoculated to *S. montanus*. A host provenance effect has been reported previously for the mistletoe *Psyttacanthus calyculatus* (Loranthaceae) thriving on five different host species (Lara et al. 2009). We do not have a clear explanation for this phenomenon; the properties of *T. verticillatus* seeds of different provenances deserve further investigation. Furthermore, seeds of *T. verticillatus* from *S. montanus* showed lower mortality than seeds from *F. imbricata* when inoculated to *S. montanus*, consistent with higher performance of mistletoe seeds on the host species of provenance (Clay et al. 1985, Rödl & Ward 2002),

Seeds of *T. verticillatus* germinated to similar extent when the fruit exocarp was manually removed and when defecated by *M. thenca*. Successful germination of seeds whose exocarp had been manually removed was also found for *T. aphyllus* (Gonzáles et al. 2007) although it was previously reported that such seeds needed to pass through the digestive tract of *M. thenca* to start germination (Soto-Gamboa & Bozinovic 2002). Likewise, adherence was also greater and mortality lower for bird-defecated seeds than for manually peeled seeds. It is likely that differences between performance of fruit whose exocarp was manually removed and when defecated by *M. thenca* may have been greater had fasting time been shorter (Murphy et al. 1993). At any rate, it may be expected that survival in the cross inoculation experiment would have been greater if bird-defecated seeds had been used.

This study is an approximation to factors affecting host use by a hemiparasitic plant, an issue which has seldom been addressed in the literature (Marquardt & Pennings 2010). The results showed that incidence and intensity of parasitic infection by *T. verticillatus* in YLNS was disproportional to the availability and appearance of the three co-occurring hosts, *S. montanus* being the least abundant and less apparent host but that with the highest incidence and intensity of infection. Thus, *S. montanus* appears as the preferred host for *T. verticillatus* at YLNS. Several features of the system may account for the effect: perching opportunities for avian dispersers, accessibility to water, morphological compatibilities between host and mistletoe and germination rate and mortality of seeds. A more detailed study evaluating the behaviour of avian dispersers in relation to the three available hosts at the study site, and the effect on mistletoe development of features related to nutritional, morphological and biochemical mistletoe-host compatibility would be welcome.

ACKNOWLEDGEMENTS

The authors thank Amparo Bruckner and Milton Fernández for their suggestions and comments, to Luis F. Aguirre for his valuable advice, and to two anonymous reviewers for their generous criticisms and suggestions. We thank the authorities administering the Yerba Loca Nature Sanctuary for allowing the completion of this study. Financial support was provided by the Latin American Network for Research on Bioactive Natural Compounds (LANBIO), the International Foundation for Science (IFS, grant 4356-1 to A. J. T.) and the National Fund for Scientific and Technological Development of Chile (FONDECYT, grant 1080248 to H. M. N.).

REFERENCES

Attsat P. R. (1977) The insect herbivore as a predictive model in parasitic seed plant biology. Am. Nat. 111, 579-586.

Aukema J. E. (2004) Distribution and dispersal of desert mistletoe is scale-dependent, hierarchically nested. Ecography 27, 137-144.

Aukema J. E. & Martínez del Río C. (2002a) Variation in mistletoe seed deposition: effects of intra-and interspecific host characteristics. Ecography 25, 139-144.

Aukema J. E. & Martínez del Río C. (2002b) Where does a fruit-eating bird deposit mistletoe seeds? Seed deposition patterns and an experiment. Ecology 83, 3489-3496.

Besag J. E. & Gleaves J. T. (1973) On the detection of spatial pattern in plant communities. B. Int. Statist. Inst. 4, 153-158

Cabezas N. J. (2007) Efecto del hospedero sobre la composición de metabolitos secundarios del hemiparasito *Tristerix verticillatus* (Loranthaceae) y sobre sus interacciones con insectos. PhD Thesis, University of Chile.

Clay K., Dement D. & Rejmanek M. (1985) Experimental evidence for host races in mistletoe (*Phoradendron tomentosum*). Am. J. Bot. 72, 1225-1231.

Dzerefos C. M., Witkowski E. T. F. & Shackleton C. M. (2003) Host-preference and density of woodrose-forming mistletoes (Loranthaceae) on savanna vegetation, South Africa. Plant Ecol. 167, 163-177.

Gajardo R. (1994) La vegetación natural de Chile. Clasificación y distribución geográfica. Editorial Universitaria, Santiago, Chile. 165pp.

Gonzáles W. L., Suárez L. H. & Medel R. (2007) Outcrossing increases infection success in the holoparasitic mistletoe *Tristerix aphyllus* (Loranthaceae). Evol. Ecol. 21, 173-183.

Huyse T., Poulin R. & Théron A. (2005) Speciation in parasites: a population genetics approach. Trends Parasitol. 21, 469-475.

Kawecki T. J. & Ebert D. (2004) Conceptual issues in local adaptation. Ecol. Lett. 7, 1225-1241.

Kelly C. K., Venable L. & Zimmerer K. (1988) Host specialization in *Cuscuta costaricensis*: an assessment of host use relative to host availability. Oikos 53, 315-320.

Krebs C. J. (1999) Ecological Methodology. Addison-Wesley Longman, Menlo Park, California.

Kuijt J. (1969) The Biology of Parasitic Flowering Plants. University of California Press. California. 246 pp.

Kuijt J. (1988) Systematic Botany Monographs. Revision of *Tristerix* (Loranthaceae). The American Society of Plant Taxonomists. 19, 61 pp.

Ladley J. J. & Kelly D. (1996) Dispersal, germination and survival of New Zealand mistletoes (Loranthaceae): dependence on birds. New Zeal. J. Ecol. 20, 69-79.

Ladley J. J., Kelly D. & Norton D. A. (1997) A guide to hand-planting of New Zealand mistletoes (Loranthaceae). New Zeal. Bot. Soc. Newslett. 49, 16-19.

Lamont B. (1983) Germination of mistletoes. In: The biology of mistletoes (eds. M. Calder & P. Bernhardt) pp. 129–143. Academic Press, Sidney.

Lara C., Pérez G & Ornelas J.F. (2009) Provenance, guts, and fate: field and experimental evidence in a host-mistletoe-bird system. Ecoscience 16, 399-407.

Marquardt E. S. & Pennings S. C. (2010) Constraints on host use by a parasitic plant. Oecologia 164, 177-184.

Marvier M. A. & Smith D. L. (1997) Conservation implications of host use for rare parasitic plants. Conserv. Biol. 11, 839-848.

Medel R., Botto-Mahan C., Smith-Ramírez C., Méndez M., Ossa C., Caputo L. & Gonzáles W. (2002) Historia natural cuantitativa de una relación parásito-hospedero: el sistema *Tristerix*-cactáceas en Chile semiárido. Rev. Chil.Hist. Nat.75, 127-140.

Medel R., Vergara E., Silva A. & Kalin-Arroyo M. (2004) Effects of vector behavior and host resistance on mistletoe aggregation. Ecology 85, 120-126.

Murphy S. R., Reid N., Yan Z. G. & Venables W. N. (1993) Differential passage time of mistletoe fruits through the gut of honeyeaters and flowerpeckers - effects on seedling establishment. Oecologia 93, 171-176.

Norton D. A. & Carpenter M. A. (1998) Mistletoes as parasites: host specificity and speciation. Trends Ecol.Evol.13, 101-105.

Norton D. A. & De Lange P. J. (1999) Host specificity in parasitic mistletoes (Loranthaceae) in New Zealand. Funct. Ecol.13, 552-559.

Norton D. A. & Ladley J. J. (1998) Establishment and early growth of *Alepis flavida* in relation to *Nothofagus solandri* branch size. New Zeal. J.Bot. 36, 213-217.

Norton D. A., Ladley J. J. & Sparrrow A. D. (2002) Host provenance effects on germination and establishment of two New Zealand mistletoes (Loranthaceae). Funct. Ecol.16, 657-663.

Pennings S. C. & Callaway R. M. (2002) Parasitic plants: parallels and contrasts with herbivores. Oecologia 131, 479-489.

Powell G. R. & Norton D. A. (1994) Contrasts in crown development of the mistletoes *Alepis flavida* (Hook. f.) Tiegh. and *Peraxilla tetrapetala* (L. f.) Tiegh. (Loranthaceae) parasitic on *Nothofagus solandri* (Hook. f.) Oerst., Craigieburn Ecological District, New Zealand. NZ J.Bot. 32, 497-508.

Press M. C. & Phoenix G. K. (2005) Impacts of parasitic plants on natural communities. New Phytol. 166, 737-751.

Rödl T. & Ward D. (2002) Host recognition in a desert mistletoe: early stages of development are influenced by substrate and host origin. Funct. Ecol. 16, 128-134.

Roxburgh L. & Nicolson S. W. (2005) Patterns of host use in two African mistletoes: the importance of mistletoe-host compatibility and avian disperser behaviour. Funct. Ecol. 19, 865-873.

Runyon J. B., Mescher M. C. & De Moraes C. M. (2006) Volatile chemical cues guide host location and host selection by parasitic plants. Science 313, 1964-1967.

Sargent S. (1995) Seed fate in a tropical mistletoe: the importance of host twig size. Funct. Ecol. 9, 197-204.

Sheskin D. J. (2000) Handbook of Parametric and Nonparametric Statistical Procedures. Chapman & Hall/CRC, Boca Ratón.

Siegel S. & Castellan, Jr. N. J. (1988) Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, London.

Soto-Gamboa M. & Bozinovic F. (2002) Fruit disperser interaction in a mistletoe-bird system: a comparison of two mechanisms of fruits processing on seed germination. Plant Ecol. 159, 171-174.

Troncoso A. J., Cabezas N. J., Faúndez E. H., Urzúa A. & Niemeyer H. M. (2010) Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. Oecologia 162, 413-425.

Watson D. M. (2001) Mistletoe – a keystone resource in forests and woodlands worldwide. Ann. Rev. Ecol. Evol. Syst. 32, 219-249.

Watson D. M. (2009) Determinants of parasitic plant distribution: The role of host quality. Botany 87, 16-21.

CAPITULO 2

Efectos ascendentes del hospedero sobre las interacciones entre un muérdago y sus polinizadores y herbívoros

(Host effects on the interactions between a mistletoe and its pollinators and herbivores: a network analysis)

Abstract Interaction norm is the change in the outcome of interspecific interactions along spatial, temporal and ecological gradients. Both mutualistic (e.g., pollination) and antagonistic (e.g., herbivory) interactions have shown to be quite flexible under changing conditions. The aim of the present study is to determine the interaction norms between the mistletoe *T. verticillatus* and its pollinators and herbivores when the former parasitises three co-occurring host species, and to assess the relationship between such interactions and observed fruit production. We demonstrated that although the three hemiparasite-host systems (HHS) are exposed to the same diversity of pollinators and herbivores within the study area, they were associated to subsets of the community of pollinators and herbivores; these subsets differed in their diversity, measured in terms of species richness, evenness and heterogeneity, and their interaction network characteristics, such as interaction strength; within HHS, differences were found in the patterns of resource use by herbivores and pollinators. Moreover, interaction strength of herbivory but not pollination was also influenced by the local abundances of HHS. Although fruit production did not differ between HHS, it was positively affected by total pollinator visits. Taken together, data shows that host species structure the pollinator and herbivore communities giving rise to three distinct subsets associated to the three HHS that differ in their diversity and network structure.

Keywords Host-parasite interactions • Pollinator network • Herbivore network • Quantitative bipartite network • Loranthaceae • *Tristerix verticillatus*

Introduction

The change in the outcome of interspecific interactions (i.e., sign, directionality and strength) under variable environments is referred to as the interaction norm (Thompson 1988). Such variable environments may include changes along spatial and temporal gradients, and also changes in ecological phenomena such as coexistence and/or interaction with other species. In mutualistic interactions such as pollination, heterospecific floral neighbourhoods affect pollinator arrival, pollen deposition and even reproductive success in a wide range of species (e.g., Johnson et al. 2003; Troncoso and Vargas 2004; Dietzsch et al. 2011; Duffy and Stout 2011). In antagonistic interactions such as herbivory, host species may exert a bottom-up influence on the interaction between their herbivores and upper trophic level species (e.g., predators and herbivore mutualists) and even become determinant of the diversity of herbivore and predator species at the community level (Crutsinger et al. 2006; Bukovinszky et al. 2008; Mooney and Agrawal 2008; Poelman et al. 2009).

Network analysis has been developed as a tool to address how trophic relationships, such as plant-pollinator and plant-herbivore interactions, shape ecological communities (e.g., Pimm 1982; Proulx et al. 2005; Bascompte et al. 2006; Montoya et al. 2006; Santamaría and Rodríguez-Gironés 2007; Vázquez et al. 2009a). The use of network analyses for the study of plant-animal interactions has advanced our understanding of community-wide patterns (Bascompte et al. 2006; Bascompte and Jordano 2006, 2007).

Bottom-up effects mediated by host plants with cascading effects on upper trophic levels in systems where the "herbivore" is actually a plant (i.e., a parasitic plant) has occasionally been addressed. For instance, the lupine and grass hosts of the hemiparasite *Castilleja indivisa* (Orobanchaceae) affect pollinator visits and herbivore attack to the hemiparasite, with an overall effect on the hemiparasite's lifetime seed set; differential uptake of alkaloids from the host effect is responsible for the effect since parasitic plants that grow on high-alkaloid content hosts are less attacked by herbivores, and also receive higher pollinator visits, whereas the opposite occurs when *C. indivisa* individuals grow on hosts with low alkaloid content (Adler 2000).

Host plants can also affect the volatiles emitted by their parasitic plants. Such is the case of the mistletoe *Tristerix verticillatus* (Loranthaceae) which exhibits a host-related chemical polymorphism in volatile organic compounds (VOC's) emitted from their flowering branches when it parasitises three co-occurring host species (*Schinus montanus*, *Fabiana imbricata* and *Berberis montana*). These VOC blends differ qualitatively and quantitatively (for example, compounds known to attract or repel some insect groups are unevenly represented in the VOC mixtures emitted by mistletoes associated to different hosts); thus, as expected, such host-mediated VOC chemotypes differ in the total number of pollinator visits they receive and in the visitation rates by some specific insect pollinators (Troncoso et al. 2010). In addition to this host-mediated VOC polymorphism, the translocation of isoquinoline alkaloids from the host *B. montana* to the mistletoe *T. verticillatus* has been reported (Cabezas et al. 2009), although its relationship with pollination and herbivory levels has not been fully evaluated.

The aim of the present study is to determine the interaction norms (changes in the strength of interactions) for the mistletoe *T. verticillatus* and its pollinator and herbivore species when the mistletoe parasitises the three above-mentioned co-occurring hosts. We hypothesise that, within the mistletoe population, the three hosts exert an influence on the interactions that the mistletoe maintains with its pollinators and herbivores. We predict that the diversity and networking characteristics of pollinators and herbivores will differ between the three hemiparasite-host systems (HHS).

Materials and methods

Place and period of study

The study was conducted in an area of ca. 45 hectares of the Villa Paulina sector in the Yerba Loca Nature Sanctuary (YLNS), 70 km northeast of Santiago, Chile (33.31°S; 70.32°W; 1,900 - 2,100 m elevation range). The vegetation is mainly composed of grasses, cushion plants and small bushes (Gajardo 1994). *Tristerix verticillatus* (Loranthaceae), a shrubby hemiparasite present in central Chile, south-eastern Bolivia and north-western Argentina (Kuijt 1988), parasitises three host species within the YLNS: *Schinus montanus* (Anacardiaceae), *Fabiana imbricata* (Solanaceae) and *Berberis montana*

(Berberidaceae); for operational purposes of the study, the three HHS will be referred to as TS, TF and TB, respectively. *Tristerix verticillatus* individuals may produce several hundred flower buds that develop from December to March, bloom from February to April, and set fruit from mid-October to December. The flowering and fruiting periods of *T. verticillatus* do not overlap with those of its three hosts; hence, the effect of hosts on the mistletoe's interactions with pollinators is indirect. Data was gathered during 2008 and the beginning of 2009.

Following Huyse et al. (2005), an infrapopulation is made up by all *T. verticillatus* individuals parasitising a single host individual. The number of infrapopulations of TS, TF and TB, their total volume, and the total number of inflorescences on each HHS in the study area differed significantly between hosts (Lemaitre et al. 2012; Saldías et al. 2012). Hence, network analysis was performed both at *per capita* and population levels in order to take into account the possible influence of abundance on the symmetry of the strength of the interactions between mistletoes and their pollinators and herbivores (Vásquez et al. 2007).

Pollinator and herbivore diversity

All infrapopulations of the three HHS in the study area (N = 176) were georeferenced and identified with a unique code. Forty-one infrapopulations (TS = 17, TF = 17, and TB = 7) were chosen after a semi-structured random procedure which took into account distribution, size and exposure to sun of the infrapopulations. For each infrapopulation, three mistletoe twigs were chosen haphazardly and monitored from flower bud formation, throughout the flowering season and until the end of the fruiting season. The number of flower buds, blooming flowers, developing fruits, and mature fruits on each focal twig along the year of study were recorded. These numbers were used to assess putative effects of the herbivore and pollinator communities on final fruit production in the focal twigs.

During the flowering season, pollinator visits (visitation frequencies) to the focal twigs and types of pollinator (species richness) were monitored. Flower visitation (number of visits and visitor species) to each evaluation unit was monitored during seven 10-min observation periods; these periods were randomly sorted between days and time intervals between 0600 and 1900 hours. Sorting was performed in such a way that no focal twig was observed more than once at any given day or time of the day. All data was collected in days with similar weather conditions; observations were not performed on cloudy days.

Monthly visits (February to November 2008) were performed to quantify the types of herbivory present (herbivore richness) on the flower buds and mature flowers (flower herbivory), on the leaves (leaf herbivory), and on fruits produced (fruit herbivory) on the focal twigs (Table 1). The final value for a given herbivory type and twig was the maximum value attained along the year; this value was recorded as a measure of the strength of the interaction.

Data analysis

Pollinator diversity was analysed in terms of species richness (number of species), evenness (representativeness of each species in relation to its abundance), and heterogeneity (structure of the pollinator community in relation to its species richness and abundance) (Krebs 1999). For the comparison of species richness, the rarefaction method (Magurran 2003) was used for each HHS with number of observations as an analogue for number of specimens. Pollinator evenness of each HHS was compared through the Buzas and Gibson's index. Finally, rank/abundance curves (qualitative comparison) and the reciprocal of Simpson's index (quantitative comparisons) were used to compare pollinator heterogeneity among HHS (Krebs 1999).

Since very few herbivore taxa could be identified and herbivore damage signals were more traceable than herbivore species themselves, different types of herbivory were distinguished and treated as separate entities for the diversity analysis. Type of herbivory richness, evenness and heterogeneity indexes were calculated and compared across HHS.

All diversity parameters (for pollinators and herbivory types) were calculated and statistically compared through a bootstrap procedure with 1000 iterations using the PAST v.2.12 software (Hammer et al. 2001).

Table 1. Types of herbivory found in *T. verticillatus* on the three host species.

Code	Herbivory type (putative herbivore)	Visual signal	Method of calculation of intensity
L1	*Mathania leucothea* and other chewing insects	Presence and consumption of leaves	Area consumed / Total leaf area
L2	*Aphis craccivora* and other sap-feeding insects	Presence	Size of colonies
FL1	*Mathania leucothea* and other chewing insects	Presence and consumption of flower buds	Proportion of bud consumed
FL2	*Aphis craccivora* and other sap-feeding insects	Presence	Size of colonies
FL3	*Camponotus chilensis* ants and other nectar robbers	Presence and holes at the base of the flowers	Size of holes
FR1	Sap-feeding scale insects	Presence	Number of fruits with scales per infrutescence
FR2	Unidentified animal	Presence and consumption of fruit tissue	Proportion of fruit consumed
FR3	White walking mites	Presence	Number of fruits with mites per infrutescence
FR4	Red mite webs with fruit abortion	Presence	Number of fruits with mite webs per infrutescence
FR5	Leaf miner	Galleries underneath the fruit cuticle	Number of fruits with galleries per infrutescence
FR6	Unknown 1	Black dots (secondary damage)	Number of fruits with dots per infrutescence
FR7	Unknown 2	Brown spots (secondary damage)	Number of fruits with spots per infrutescence
FR8	Leaf roller	Fruit tissue partially curled	Number of fruits with roller damage per infrutescence

For the interaction network analyses, quantitative interaction matrices were constructed with the interaction frequencies of the j^{th} pollinator species (N = 20) and the i^{th} HHS (N = 3) for the pollination network, and with the degree of herbivory of the j^{th} herbivory type (N = 13) and the i^{th} HHS for the herbivory type network. Based on the pollinator and herbivore interaction matrices, a species-level analysis allowed the calculation of interaction dependence and interaction strength, and a network-level analysis allowed the calculation of generality, vulnerability and interaction strength asymmetry. The interaction norms between *T. verticillatus* and its pollinators and herbivores were determined; the networks constructed are meaningful for *T. verticillatus* only, since they do not represent the complete plant and animal community of the study site. Network analyses were performed with the bipartite package (Dormann et al. 2009) developed for the R package (version 2.14.1, R Development Core team 2011). To estimate significance of the species-level and network-level parameters, the indexes obtained were compared with a set of indexes obtained from random networks built with null model 1 (r2dtable); the chosen model maintained the dimensionality and connectance of the webs. Comparisons were performed using the t-test iterative procedure of the R package (null.t.test) with 100 replicates. These analyses were represented graphically in two ways: i) as a grid on which intensity of pairwise interactions were depicted with different levels of grey, darker referring to more intense interactions, and ii) as a bipartite graph on which the lower trophic level (HHS) at the bottom and the higher trophic level (pollinators or herbivores) at the top were connected by lines whose widths represent the intensities of the interactions.

The interaction network analyses were performed in two ways: i) the intensities of the interactions with pollinators and herbivores were averaged accross samples in order to determine the interaction strength experienced by an average mistletoe individual of each HHS (*per capita* level); and ii) the averages of the intensities of the interactions with pollinators and herbivores were multiplied by the local abundance of each HHS in order to quantify the level of interaction strength experienced by each HHS (population level). The abundance of HHS was estimated, in the case of pollinators, as the total number of flowers in the study area; in the case of herbivores, the abundance of HHS was estimated as the total volume of mistletoe infrapopulations in the study area.

Finally, at the end of the fruiting season, total fruit production was recorded on each monitored twig. Comparison between HHS was performed with one-way ANOVA.

In order to test the effect of total pollinator visits and accumulated herbivory on focal twig fruit production success (number of fruits / number of flowers per twig), a forward stepwise general regression model (GRM) analysis was performed. These analyses were performed using Statistica 8 (Statsoft 2007).

Results

Interactions with pollinators

Tristerix verticillatus was visited by 20 species that actively contacted the reproductive parts of flowers (18, 14 and 12 species for TS, TF and TB, respectively). Eighteen of the 20 visitor species were insects of the orders Lepidoptera, Hymenoptera and Diptera; the remaining two were the hummingbirds *Sephanoides sephaniodes* and *Oreotrochilus leucopleurus* (Figure 1). The species richness of TS was significantly higher than that of TF and TB, as seen through the rarefaction curves (Fig. 2A); moreover, when looking at each HHS separately (Figs. 2B, 2C, and 2D), it is apparent that the three rarefaction curves reached an asymptote, suggesting that an increase in sampling effort (additional recordings of flower visits) would not have increased significantly the species richness on each HHS (significance is inferred from the confidence intervals).

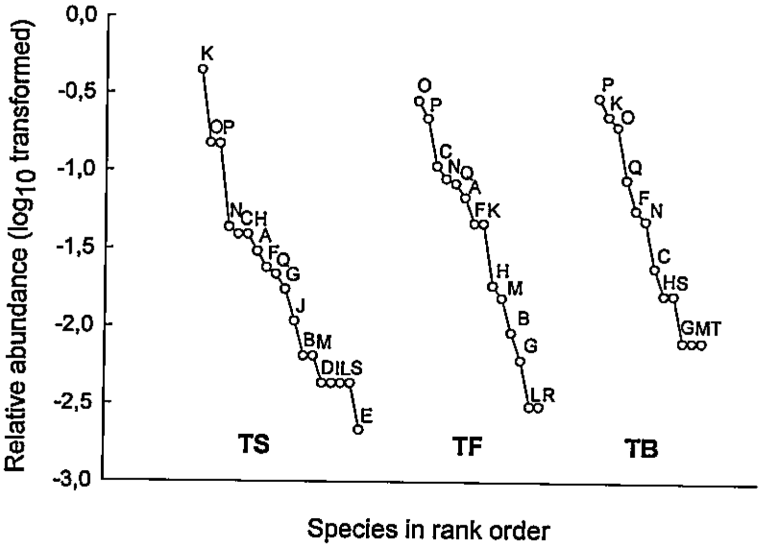

Figure 1. Rank-abundance curves representing the changes in heterogeneity in pollinator visits to each HHS. Pollinator taxa identified through capital letters as follows: *Sephanoides sephaniodes* (A), *Oretrochilus leucopleurus* (B), *Tatochila mercedis* (C), *Vanessa carye* (D), *Homoeonympha boisduvali* (E), *Episyrphis* sp. (F), Tachinidae sp. 1 (G), *Vespula germanica* (H), Vespidae sp. 2 (I), Ichneumonidae sp. 1 (J), *Bombus dahlbomi* (K), *Bombus terrestris* (L), *Apis mellifera* (M), *Megachile* sp. (N), Diptera sp. 1 (O), Diptera sp. 2 (P), Halictidae sp. 1 (Q), Halictidae sp. 2 (R), Lepidoptera sp. 1 (S), *Yramea cytheris* (T).

Rank-abundance curves showed different situations depending on the pollinator: i) some pollinators changed considerably in relative abundance between HHS; for example, *Bombus dahlbomi* (K, fig. 1) was the dominant pollinator of TS, shared dominance with two diptera species on TB, and was in the mid-dominance range in TF; ii) some species ranked similarly in abundance between HHS; for example, two dominant unidentified dipteran species (O and P, fig. 1) and the less abundant bees *Megachile* sp. and *Apis mellifera* (N and M, fig. 1) visited the three HHS to similar extents; iii) some species visited only one of the HHS; for example, the Lepidopteran *Vanessa carye* (B, fig. 1) and an Ichneumonidae sp. (J, fig. 1); and iv) the two species of hummingbirds (A and B, fig. 1), which were rather infrequent and did not occur at all in TB, in spite of *T. verticillatus* having been described as an ornithophilic species (Kuijt 1988). Diversity indexes for pollinators differed between the three HHS (Table 2). Species richness followed the order TS > TF > TB; evenness and heterogeneity indexes followed the order TF > TB > TS (Table 2).

Interaction networks obtained for pollinators, both at *per capita* and population levels, are shown in figure 3. Comparisons in table 3 showed that the structures of the plant-pollinator networks were not due to random sampling. Plant-pollinator networks showed positive values of interaction strength asymmetry at the *per capita* and population levels; hence, pollinators showed some degree of selectivity towards HHS. Furthermore, on the average HHS's effectively interacted with an average of seven out of the 20 pollinator species (vulnerability, table 3), while each pollinator species interacted with an average of two HHS (generality, table 3). This pattern was observed both at *per capita* and population levels. Interaction strength was affected by HHS ($F = 29.775$, $df = 2$, $P < 0.001$) and by abundance ($F = 0.0848$, $df = 1$, $P < 0.001$), but not by the interaction of HHS and abundance ($F = 1.840$, $df = 2$, $P = 0.771$); interaction strengths followed the order: TS > TF > TB both at the *per capita* and population levels (Fig. 4).

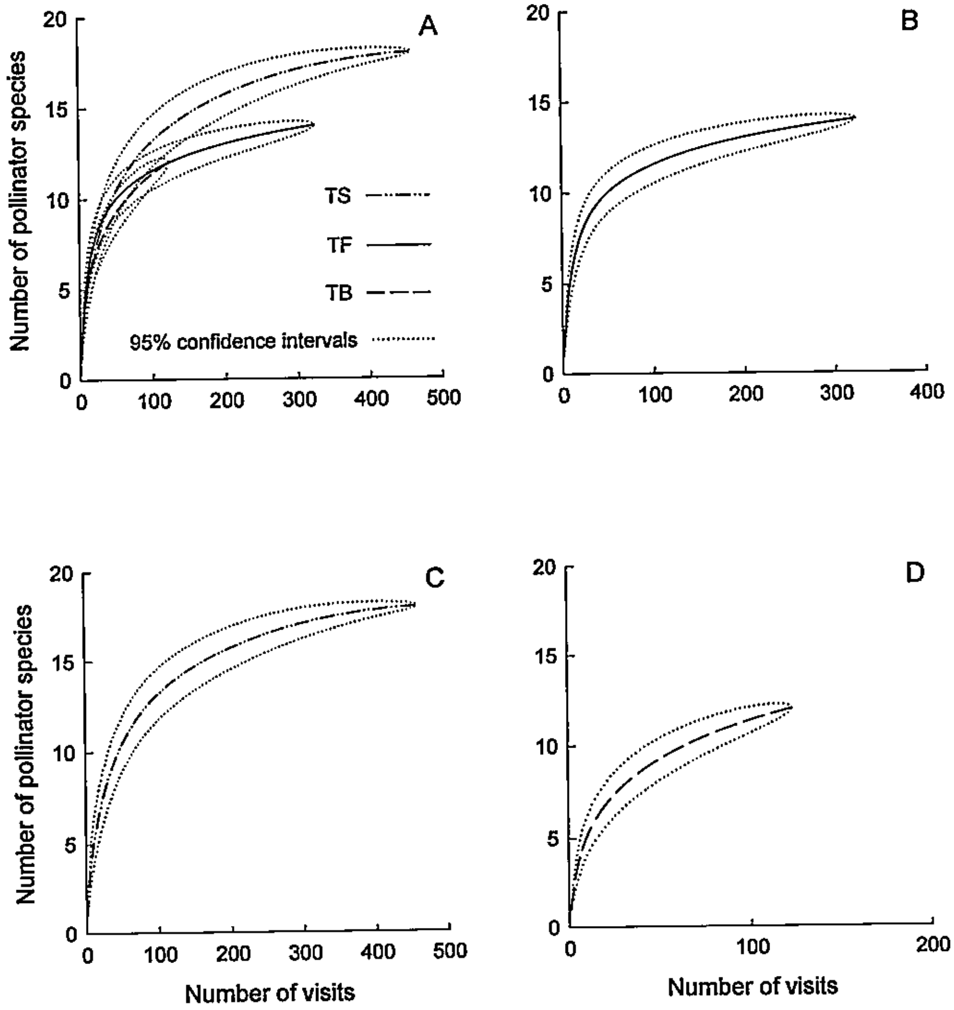

Figure 2. Rarefaction curves for the three HHS together (A) and species-accumulation curve for each HHS separately (B, C, and D).

Table 2. Diversity indexes for pollinators and herbivores of *T. verticillatus* on the three host species. Different letters in each diversity test indicate differences between groups after a permutation test ($P = 0.001$).

Diversity index	*Tristerix verticillatus* infecting three hosts		
	S. montanus	*F. imbricata*	*B. montana*
Pollinators			
S (species richness)	18 a	14 b	12 c
Chao-1 estimator (±SD)	18.125 (±0.354)	16.0 (±3.010)	14.25 (±2.813)
$e^{H'}/S$ (Buzas & Gibson's evenness)	0.367 c	0.566 a	0.549 b
1/D (reciprocal of Simpson's index *D*)	4.010 c	6.064 a	5.120 b
Herbivores			
S (herbivory type richness)	13 a	12 b	11 c
$e^{H'}/S$ (Buzas & Gibson's evenness)	0.828 a	0.661 c	0.722 b
1/D (reciprocal of Simpson's index *D*)	9.940 a	6.610 b	6.570 c

Interactions with herbivores

Two types of leaf herbivory, three types of flower herbivory and eight types of fruit herbivory could be distinguished (Table 1). All types of herbivory occurred in the three HHS, albeit to different extents. Thus, diversity indexes for type of herbivory differed between the three HHS. Type of herbivory richness followed the order TS > TF > TB, evenness index followed the order TS > TB > TF, and heterogeneity index followed the order TS > TB > TF (Table 2).

Interaction networks obtained for herbivore types, both at *per capita* and population levels, are shown in figure 5. Comparisons in table 3 show that the structures of the plant-herbivore networks were not due to random sampling. Plant-herbivore type networks showed a negative value of interaction strength asymmetry at the population level and a positive value at the *per capita* level (Table 3); hence, herbivores show a certain degree of selectivity towards HHS as individuals which decreases at the population level. Furthermore, on the average HHS's effectively interacted with an average of 8 to 9 out of the 13 herbivory types (vulnerability, table 3), while each herbivory type interacts with an average of two HHS (generality, table 3). Interaction strength was affected by HHS (F = 21.597, df = 2, P < 0.001) and by the interaction of HHS and abundance (F = 12.347, df = 2, P < 0.001), but not by abundance alone (F = 0.000, df = 1, P = 1.000); interaction strengths followed the order TS > TF > TB at the population level (Fig. 4).

Comparison between plant-pollinator and plant-herbivore networks

The degree of dependency calculated through the species-level analysis of the interaction networks was used as a proxy of the intensity of use of each HHS by pollinators and herbivores. The intensity of use of TS did not differ between pollinators and herbivores (type of interaction effect: F = 0.97, df = 1, P = 0.33), and increased when escalating from *per capita* to population level (abundance effect: $F = 10.20$, $df = 1$, $P = 0.002$) for both pollinators and herbivores (interaction of effects: $F = 0.59$, $df = 1$, $P = 0.45$). In the case of TF, the intensity of use by herbivores and pollinators did not differ at any level of comparison (type of interaction, abundance, and their interaction of effects: $F = 0.87$, $df = 1$, $P = 0.36$; $F = 0.000$, $df = 1$, $P = 0.99$; and $F = 0.20$, $df = 1$, $P = 0.66$, respectively). Finally, the intensity of use of TB differed between pollinators and herbivores ($F = 6.54$, $df = 1$, $P = 0.013$), and they decreased when escalating from *per capita* to population level ($F = 12.41$, $df = 1$, $P < 0.01$) for both types of interaction (interaction of effects: $F = 1.31$, $df = 1$, $P = 0.26$).

Fruit production

No differences were found in fruit production success (number of fruits / number of flowers per twig) by the three HHS during the period of study ($F = 1.082$, $df = 2$, $P = 0.345$). However, the GRM analysis showed that fruit production success was positively affected by total pollinator visits and was not affected by either total herbivory or HHS (overall model: $F = 11.30$, $df = 1$, multiple $R^2 = 0.24$, $P = 0.002$; partial regression coefficient for total pollinator visits = 0.04, $t = 3.36$, $P = 0.002$).

Table 3. Network-level parameters for the quantitative bipartite networks of *T. verticillatus* on three host species, and its pollinators and herbivores.

Network-level parameters	**Observed value**	**Null model mean**	***t***	***P***
Plant-pollinator network				
At population level				
Interaction strength asymmetry	0.39	0.28	-399.24	<0.001
Generality	1.73	1.85	-26.11	<0.001
Vulnerability	6.90	7.62	87540	<0.001
At *per capita* level				
Interaction strength asymmetry	0.30	0.24	-27.02	<0.001
Generality	2.36	2.62	264.53	<0.001
Vulnerability	7.07	7.97	267.22	<0.001
Plant-herbivore network				
At population level				
Interaction strength asymmetry	-0.69	0.30	339.90	<0.001
Generality	1.97	1.89	-15.61	<0.001
Vulnerability	8.01	7.93	-3.60	<0.001
At *per capita* level				
Interaction strength asymmetry	0.24	0.22	-7.04	<0.001
Generality	2.60	2.90	101.28	<0.001
Vulnerability	8.90	10.00	105.11	<0.001

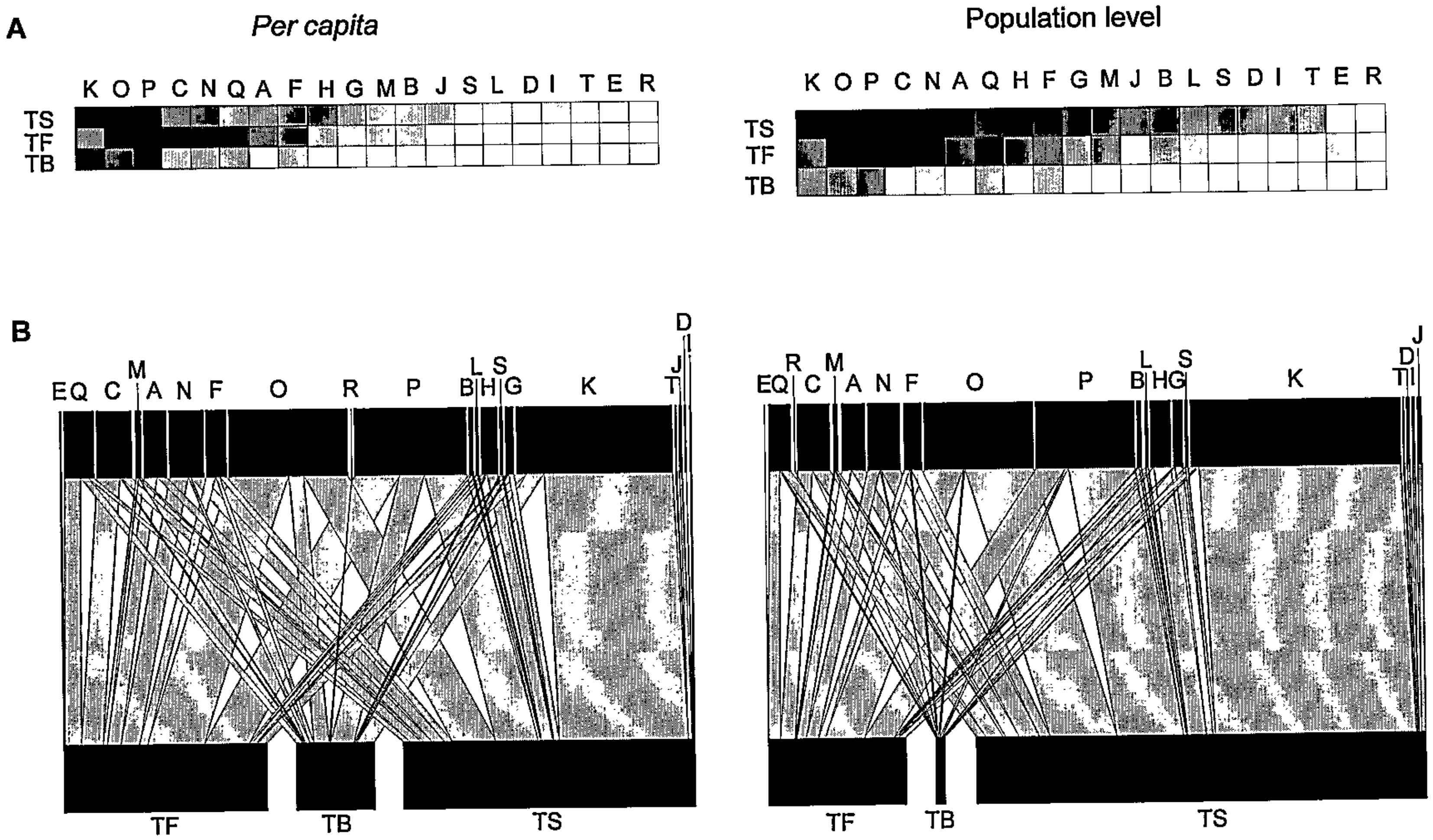

Figure 3. Pollinator – HHS web. A) Network grid where the level of grey of each cell depicts the intensity of pairwise interaction dependence between each HHS and each pollinator species. B. Quantitative bipartite network. Codes for pollinators are shown in figure 1.

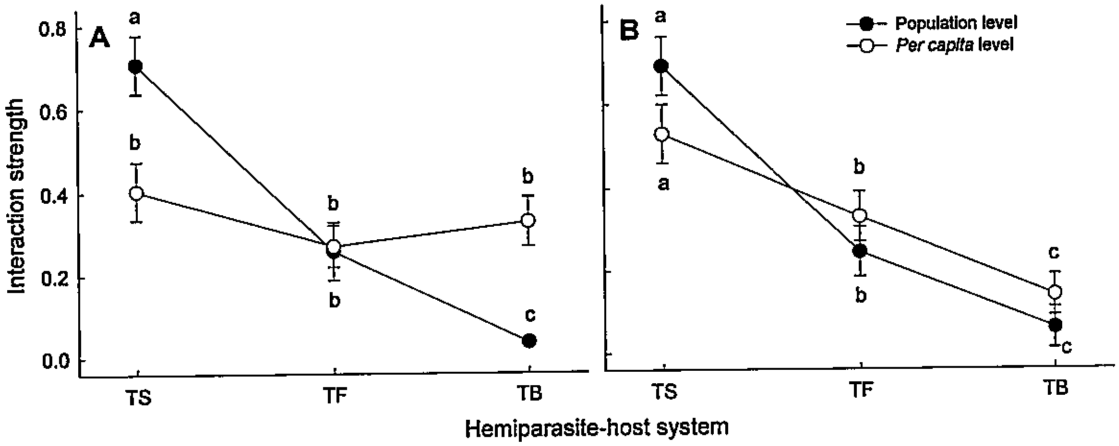

Figure 4. Interaction norms showing host and abundance effects on the interaction strengths of mistletoe-herbivore (A) and mistletoe-pollinator (B) networks.

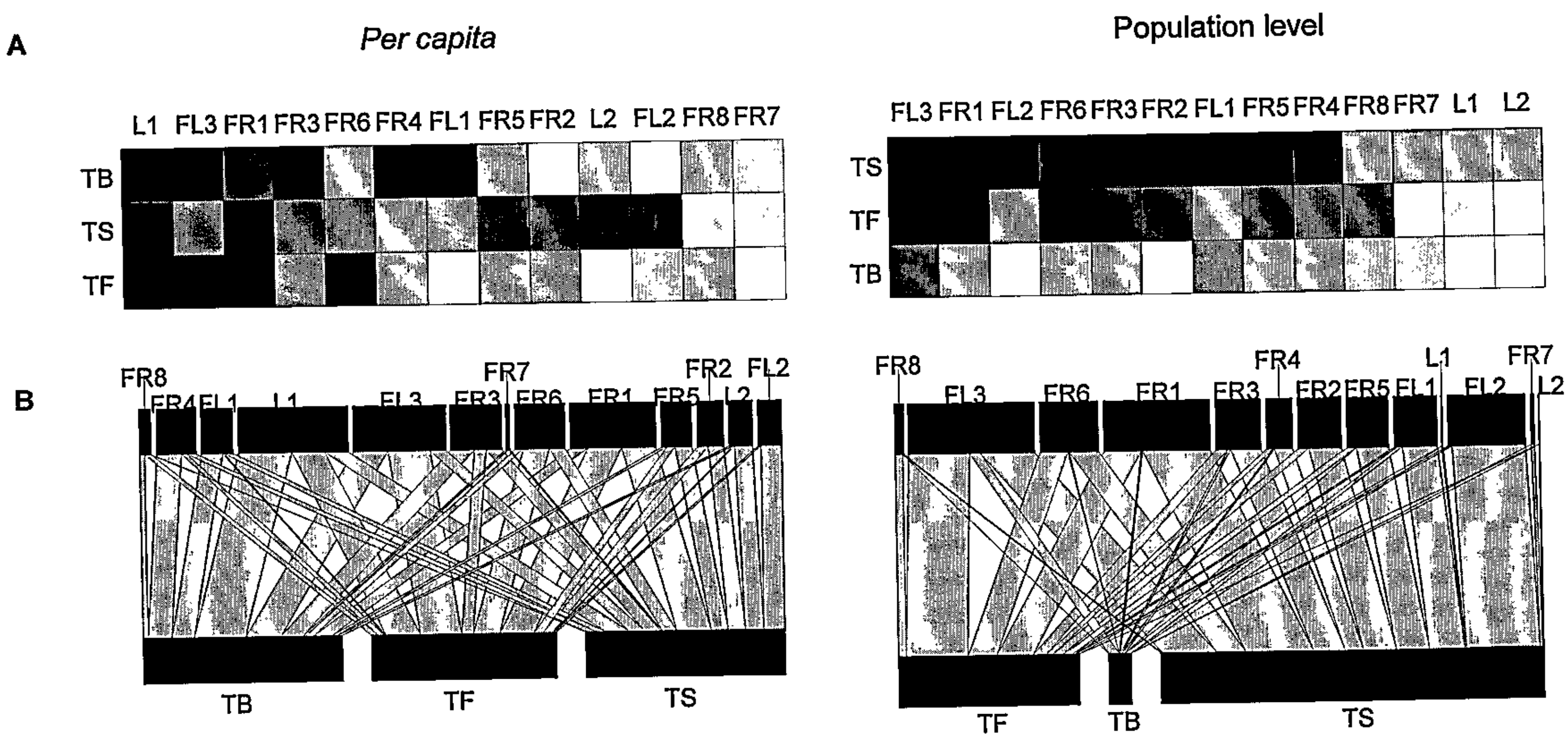

Figure 5. Herbivore – HHS web. A) Network grid where the level of grey of each cell depicts the intensity of pairwise interaction dependence between each HHS and each herbivore type. B) Quantitative bipartite network. Codes for types of herbivory are shown in table 1.

Discussion

Host species effects have been described on the abundance of pollinators and herbivores associated to a parasitic plant (Adler 2003). Similar effects have been found for *T. verticillatus* which co-occurs on three different hosts giving rise to three different HHS (Troncoso et al. 2010). Using the latter system, the present study further enquires on the diversity of pollinators and herbivores associated to the mistletoe. The three HHS were associated to subsets of the community of pollinators and herbivores; these subsets differed in their diversity, measured in terms of species richness, evenness and heterogeneity, and their interaction network characteristics, such as interaction strength; within HHS, differences were found in the patterns of resource use by herbivores and pollinators. Moreover, interaction strength of herbivory but not pollination was also influenced by the local abundances of HHS (Fig. 4). Taken together, data shows that interactions of *T. verticillatus* with pollinators and herbivores are affected by the host and give rise to interaction norms. Although fruit production did not differ between HHS, it was positively affected by total pollinator visits.

An interaction norm is traditionally expressed as a pair-wise interaction of genotype-by-genotype-by-environment (Agrawal 2001); in the present scenario, it is expressed as pollinator-by-mistletoe-by-environment and herbivore-by-mistletoe-by-environment interactions, where the environment is the host species. The present study has not only described the occurrence of interaction norms at the species level for some pollinators and herbivores (Table 2), but also at the network level (pollinator and herbivore network structures), as shown by the change in the average interaction strength between HHS (Figs. 4 and 5).

Plant-animal network structure can be influenced by species abundance (Vázquez et al. 2007, 2009b), vegetation strata (Schleuning et al. 2011), climate and habitat change (O'Connor 2009; Tylianakis et al. 2007), and resource quality (Bukovinszky et al. 2008). The combined comparison of the effects of HHS and the effect of *per capita* and population level escalation in our system brought up two main results: i) the uneven herbivore use of the three HHS at the population level was a by-product of the uneven relative abundance of the three HHS, which agrees with the well-documented influence of species abundance on the structure of interaction networks (Vásquez et al. 2007, 2009a,b); and ii) the pattern of HHS use by pollinators remained highly skewed towards TS and it was independent of TS abundance (Fig. 4 and Fig. 5 A, B). To date, flower display size, relative abundance, and HHS aggregation have been ruled out as factors to explain pollinator visits in this system, whereas VOC's emitted from the flowering branches (Troncoso et al. 2010) and nectar production (data not shown) remain as plausible causes of such skewed pollinator preference (Troncoso et al. 2010, Saldías et al. 2012).

Tristerix species produce one-ovule flowers which produce one-seeded fruits; hence, fruit production (number of fruits per inflorescence) constitutes a reliable proxy of female fitness in this species. Follow-up monitoring revealed that there were no differences in fruit production (number of fruits / number of flowers per twig) between TS, TF and TB during the period of study. On the other hand, regression analyses revealed that pollinators exerted a slight but positive influence on fruit production by *T. verticillatus* whereas herbivory had no effect on fruit production. Given the uneven levels of infection on the three host species - highest on TS (Lemaitre et al. 2011) - fruit production is more likely to be to be resource limited. However, detailed studies of intraspecific within-host competition would be desirable to elucidate this issue.

The structure of interaction networks can be differentially affected by the type of interaction selected. Mutualistic networks favour the prevalence of asymmetric specialisation whereas antagonistic networks favour symmetry and compartmentalisation (Thébault and Fontaine 2008). As a result, generalist interacting species are more frequently found in pollination networks whereas specialist interacting species are more frequently found in herbivory networks (Fontaine et al. 2009). The comparison of the intensity of use between the pollination and herbivory networks, and between *per capita* and population levels revealed that the pattern of use of the three HHS between herbivores and pollinators differed only for TB at the *per capita* level. This may result from the escalation from the population level since TB is the least abundant HHS (e.g., TS occupies an average of 10% of host volume and 96 infrapopulations occur in the study area, whereas TB occupies an average of 0.4% of host volume and 45 infrapopulations occur in the study area; Lemaitre et al. 2012, Saldías et al.

2012). Nonetheless, to elucidate if host species determine the occurrence of specialisation within the mistletoe's pollinator and herbivore networks, additional studies in other mistletoe populations and across its distribution range are desirable.

In conclusion, the interaction norms detected both at the species-level and network-level in the pollinator and herbivore communities that interact with *T. verticillatus* reflect that, although the three HHS co-occur within a small area and are thus exposed to the same pollinators and herbivores, hosts structure the existing pollinator and herbivore communities originating three distinct subsets that differ in their diversity and networking properties.

ACKNOWLEDGEMENTS

We are indebted to FONDECYT (grant 1080248 to HMN) and to the International Foundation for Science (grant 4356-1 to AJT) for financial support. We thank I. Municipalidad de Lo Barnechea for a permit to perform the studies at the Yerba Loca Nature Sanctuary.

REFERENCES

Adler LS (2000) Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. Am Nat 156:92-99

Adler LS (2003) Host species affects herbivory, pollination, and reproduction in experiments with parasitic *Castilleja*. Ecology 84:2083–2091

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321-326

Bascompte J, Jordano P (2006) The structure of plant-animal mutualistic networks. In: Pascual M, Dunne JA, (eds) Ecological Networks: Linking structure to dynamics in food webs. Oxford University Press, pp. 143-159.

Bascompte J, Jordano P (2007) Plant–animal mutualistic networks: the architecture of biodiversity. Annu Rev Ecol Evol Syst 38:567–593

Bascompte J, Jordano P, Melián CJ, Olesen JM (2003) The nested assembly of plant–animal mutualistic networks. Proc Natl Acad Sci (USA) 100:9383-9387

Bascompte J, Jordano P, Olesen JM (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. Science 312:431–433

Bukovinszky T, van Veen FJF, Jongema Y, Dicke M (2008) Direct and indirect effects of resource quality on food web structure. Science 319: 804-807

Cabezas NJ, Urzúa AM, Niemeyer HM (2009) Translocation of isoquinoline alkaloids to the hemiparasite, *Tristerix verticillatus* from its host, *Berberis montana*. Biochem Syst Ecol 35:225-227.

Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313: 966-968

Dietzsch AC, Stanley DA, Stout JC (2011) Relative abundance of an invasive alien plant affects native pollination processes. Oecologia 167: 469-479

Dormann CF, Fründ J, Blüthgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. Open Ecol J 2:7-24

Duffy KJ, Stout JC (2011) Effects of heterospecific and conespecific floral density on the pollination of two related rewarding orchids. Plant Ecol 12:1397-1406

Fontaine C, Thébault E, Dajoz I (2009) Are insect pollinators more generalist than insect herbivores. Proc Royal Soc B 276:3027-3033

Gajardo R (1994) La vegetación natural de Chile: clasificación y distribución geográfica. Editorial Universitaria, Santiago.

Hammer Ø, Harper DAT., Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electr PE4.1.4A

Huyse T, Poulin R, Théron A (2005) Speciation in parasites: a population genetics approach. Trends Parasitol 21:469-475

Johnson SD, Peter C, Nilsson LA, Ågren J (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. Ecology 84:2919-2917

Krebs CJ (1999) Ecological Methodology. Addison-Wesley Longman, Menlo Park, California.

Kuijt J (1988) Revision of *Tristerix* (Loranthaceae). Syst Bot Monogr 19:1-61

Lemaitre A, Troncoso AJ, Niemeyer HM (2011) Host preference of a temperate mistletoe: disproportional infection on three co-occurring host species influenced by differential success. Austral Ecol, in press. doi:10.1111/j.1442-9993.2011.02281.x

Magurran AE (2003) Measuring biological diversity. Wiley-Blackwell, Cowley Road, Oxford.

Mooney KA, Agrawal AA (2008) Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. Am Nat 171:E195-E205

Montoya J, Pimm SL, Solé RV (2006) Ecological networks and their fragility. Nature 442:259–264

O'Connor MI (2009) Warming strengthens an herbivore-`plant interaction. Ecology 90:388-398

Pimm SL (1982) Food webs. Chapman and Hall, London, UK

Pimm SL, Lawton JH, Cohen JE (1991) Food web patterns and their consequences. Nature 350:669-674

Poelman EH, van Dam NM, van Loon JJA, Vet LEM, Dicke M (2009) Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. Ecology 90:1863-1877

Proulx SR, Promislow DEL, Phillips PC (2005) Network thinking in ecology and evolution. Trends Ecol Evol 20:345-353

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Saldías MP, Troncoso AJ, Niemeyer HM (2012) Host-mediated bottom-up effects on pollen movement within a mistletoe population. Austral Ecol, in press

Santamaría L, Rodríguez-Gironés MA. 2007. Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? PLoS Biology 5:354-362

Schleuning M, Blüthgen N, Florchinger M, Braun J, Schaefer HM, Böhning-Gaese K (2011) Specialization and interaction strength in a tropical plant-frugivore network differ among forest strata. Ecology 92:26-36

StatSoft, Inc. (2007) STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Thébault E, Fontaine C (2008) Does asymmetric specialization differ between mutualistic and trophic networks? Oikos 117:555-563

Thompson JN (1988) Variation in interspecific interactions. Annu Rev Ecol Syst 19:65-87

Troncoso AJ, Cabezas NJ, Faúndez E, Urzúa A, Niemeyer HM (2010) Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. Oecologia 162:413-425

Troncoso AJ, Vargas RR (2004) Efecto del vecindario floral sobre la tasa de visitas por insectos a *Loasa triloba* Domb. Ex A.J. Juss. y *Loasa tricolor* Ker-Gawl en la Reserva Nacional de Rio Clarillo, Region Metropolitana, Chile. *Chloris Chilensis*, 7, http://www.chlorischile.cl (January 20th, 2011)

Tylianakis JM, Tscharntke T, Lewis OT (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. Nature 445:202-205

Vázquez DP, Blüthgen N,Cagnolo L, Chacoff NP (2009a) Uniting pattern and process in plant-animal mutualistic networks: a review. Ann Bot 103:1445-1457

Vázquez DP, Chacoff N, Cagnolo L. (2009b) Evaluating multiple determinants of the structure of mutualistic networks. Ecology 90: 2039-2046

Vázquez DP, Melián CJ, Williams NM, Blüthgen N, Krasnov BR, Poulin R (2007) Species abundance and asymmetric interaction strength in ecological networks. Oikos 116:1120-1127.

CAPÍTULO 3

Efectos ascendentes mediados por el hospedero sobre el movimiento de polen dentro de una población de muérdago

(Host-mediated bottom-up effects on pollen movement within a mistletoe population)

Abstract Bottom-up effects on tritrophic interactions through plant secondary chemistry has been extensively studied, but few studies address tritrophic systems with parasitic plants as the second trophic level (i.e. host plant-parasitic plant-pollinators). Moreover, the extent to which bottom-up host effects influence potential gene flow in parasitic plants has not been addressed to date. In the Yerba Loca Nature Sanctuary, northeast of Santiago, Chile, the hemiparasitic plant *Tristerix verticillatus* (Loranthaceae) is found as three co-occurring Hemiparasite-Host Systems (HHS) involving *Schinus montanus, Fabiana imbricata* and *Berberis montana* as hosts. We recently showed that individuals of *T. verticillatus* in these three HHS exhibit a chemical polymorphism of emitted volatile compounds and that those differences are associated with different visitation rates of insect pollinators. Mistletoe individuals parasitizing *S. montanus* were more visited than mistletoes in the other two HHS. Moreover, within the study area, *S. montanus* was parasitised more intensely than the other two hosts and higher than expected by its local availability and appearance; hence, *S. montanus* is considered the preferred host.

We hypothesise that when *T. verticillatus* parasitises the *Schinus* host; it exports and receives more pollen when compared with individuals parasitizing the other two hosts. To test this hypothesis, we examined the movement of fluorescent dyes, used as pollen-analogues, and also assessed the extent to which floral display size and flower neighbourhood were related to the patterns of fluorescent powder movement. Our results revealed that the reception of fluorescent powders differed significantly between different HHS, was independent of the donor HHS, and showed a predominant potential movement within and towards the *T. verticillatus* – *S. montanus* system. Moreover, the probability of fluorescent powder arrival was affected by the host of the receiving HHS but was independent of floral display size and flower neighbourhood of the receiving HHS. Hence, pollen movement in our study system is not panmictic;

on the contrary, it is mainly driven by the HHS (intrinsic characteristics) and not by the spatial configuration of the floral neighbourhood.

Key words: fluorescent dye, gene flow, host-parasite interaction, Loranthaceae, *Tristerix*

INTRODUCTION

Bottom-up effects mediated by differences in the secondary chemistry of host plants with cascading effects on upper trophic levels has been extensively studied in plant-herbivore-predator and plant-herbivore-mutualist systems (i.e.: Bailey et al. 2006; Bukovinszky et al. 2008; Poelman et al. 2009); however, such bottom-up effects of secondary chemistry on host plant-parasitic plant-mutualists has seldom been addressed (Adler 2000; Troncoso et al. 2010). In parasitic plants, the chemistry of the host plant affects the interactions between the parasite and its community of mutualists, such as pollinators, sometimes with consequences on the reproductive success of the parasite. Adler (2000) provides an excellent example of the latter: the hemiparasite *Castilleja indivisa* (Orobanchaceae), showed less herbivory and higher pollinator visits when it grew on hosts with high-alkaloid than with low-alkaloid content, with direct consequences on the parasite's fitness.

Plant chemistry has been recognised as a major driving force behind animal-assisted pollination (Knudsen et al. 2004; Dobson 2006; Kessler et al. 2008; Raguso 2008). In a natural sanctuary near Santiago, Chile, a single population of the mistletoe *Tristerix verticillatus* (Fig. 1) is found parasitising three co-occurring host species (*Schinus montanus*, *Fabiana imbricata* and *Berberis montana*) and is pollinated by a dozen different insect species and also, albeit to a lesser extent, by hummingbirds (Troncoso et al. 2010). This population of *T. verticillatus* exhibited chemical polymorphism of emitted volatiles, with three distinct host-related volatile phenotypes; these phenotypes differed on the presence and content of certain compounds known to attract and repel some insect groups. Such polymorphism was associated with different total number of pollinator visits (Troncoso et al. 2010), mistletoe individuals being more visited when parasitising *S. montanus* than when parasitising the other two hosts, and also different visitation rates by some insect pollinators (Troncoso et al. 2010). Moreover, within the study area, *S. montanus* was more parasitised than expected by its local availability and appearance; thus, the number of infected individuals and the hemiparasitic load (hemiparasite/host volume ratio) were higher in comparison with the other two hosts. These results suggested *S. montanus* as the preferred host over the other two (Lemaitre et al. 2011).

Based on these phenomena, and taking into account the bottom-up effect that hosts exert on mistletoe chemistry and on their community of pollinators, it may be hypothesised that individuals of *T. verticillatus* on *S. montanus* will export and receive more pollen than the other two hemiparasite-host systems. In order to test this hypothesis, we performed experiments on movement of fluorescent powders used as pollen analogues. Since pollinators are responsive to floral display size (mistletoe flowers available) and infrapopulation proximity (flowering infrapopulations of the same and different hemiparasite-host systems) (Ohashi & Yahara 2001; Caraballo-Ortiz et al. 2011), we also assessed the extent to which mistletoe floral display size and flower neighbourhood were able to predict the observed patterns of fluorescent dye movement.

METHODS

Study area

The study was conducted in the Yerba Loca Nature Sanctuary (YLNS) (33.31°S, 70.32°W, 70 km northeast of Santiago, Chile) in an area of ca. 45 hectares (sector Villa Paulina; 1,900 - 2,100 m elevation range) dominated by graminaceous and cushion plants and small bushes with hard and perennial leaves (Gajardo 1994). *Tristerix verticillatus* (Loranthaceae) is a shrubby hemiparasite present in central Chile, south-eastern Bolivia and north-western Argentina (Kuijt 1988). Within the YLNS, *T. verticillatus* is found parasitizing three host species: *Schinus montanus* (Sapindales: Anacardiaceae), *Fabiana imbricata* (Solanales: Solanaceae) and *Berberis montana* (Ranunculales: Berberidaceae), thus constituting three distinct hemiparasite-host systems (HHS: TS, TF and TB). *Tristerix verticillatus* individuals may produce several hundred flower buds that develop during the summer (December – March) and bloom from February to April (Troncoso et al. 2010); this flowering period does not overlap with those of its three host species which occur from the end of August until January, thus, the effect of hosts on the mistletoe's interactions is truly indirect. *Tristerix verticillatus* is pollinated by a dozen different insect species, particularly bees (principally *Apis mellifera, Centris nigerrima* and *Megachile* sp.) and bumblebees (mainly *Bombus dahlbomi*) and syrphid flies (*Episyrphis* spp), and also, albeit to a lesser extent, by the hummingbirds *Sephanoides sephaniodes* and *Oreotrochilus leucopleurus* (Troncoso et al. 2010). Data for this study was gathered between February and December 2009.

Floral display size of the mistletoe

Each hemiparasite infrapopulation, i.e., all individuals of the hemiparasite occurring on a given host individual (Huyse et al. 2005), was georeferenced and identified with a unique code that specified the HHS and the number of the infrapopulation. The floral display size of each infrapopulation in the study area was determined as the number of inflorescences in the infrapopulation times the mean number of flowers per inflorescence; this latter was determined from ten randomly chosen inflorescences (from ten randomly chosen mistletoe individuals) per infrapopulation. We have used number of flowers per infrapopulation as the relevant variable to assess attraction of flower visitors because it encompasses subjacent small-scale flower characteristics such as nectar and pollen rewards.

Potential movement of pollen of the mistletoe

The potential movement of pollen between *T. verticillatus* infrapopulations was estimated using fluorescent powders (Van Rossum et al. 2011; Waser & Price 1982) of different colours (BioQuip Products). Fluorescent dye powder was applied to donor infrapopulations designated on the basis of the spatial distribution of *T. verticillatus* infrapopulations in the study area (Fig. 2A), in order to balance the donor infrapopulations within low and high density areas. The areas with low and high densities of the mistletoe were identified through nearest-neighbour density analyses performed with Geographical Information Systems (GIS) using ArcView 3.2 software (ESRI 1992). Firstly, high and low density areas were mapped considering all three HHS (0-21.2 and 21.2-42.4 infrapopulations/hectare for low and high density areas, respectively); secondly, high and low density areas were mapped considering each HHS separately (TS: 0-15.8 and 15.8-31.6; TF: 0-6.6 and 6.6-13.2; TB: 0-7 and 7-14 infrapopulations/hectare, for low and high density areas, respectively). After this, donor infrapopulations were evenly distributed between high and low density areas, considering the density maps of both pooled and separated HHS (Fig. 2B).

The experiment with fluorescent powder dyes was performed during March 2009 in days of clear skies. Field observations during four consecutive nights showed the absence of nocturnal pollinators for this system. After these baseline studies, experiments were set up each night, after checking that diurnal pollinators had ceased to be active. Each night, fluorescent powder was applied on the anthers of 4 flowers in the anthesis period (recently opened flowers, as judged by the full load of pollen in the anthers and the colour of stamens; Figs. 1A and 1B) in a single hemiparasite infrapopulation of each HHS (donor infrapopulation).

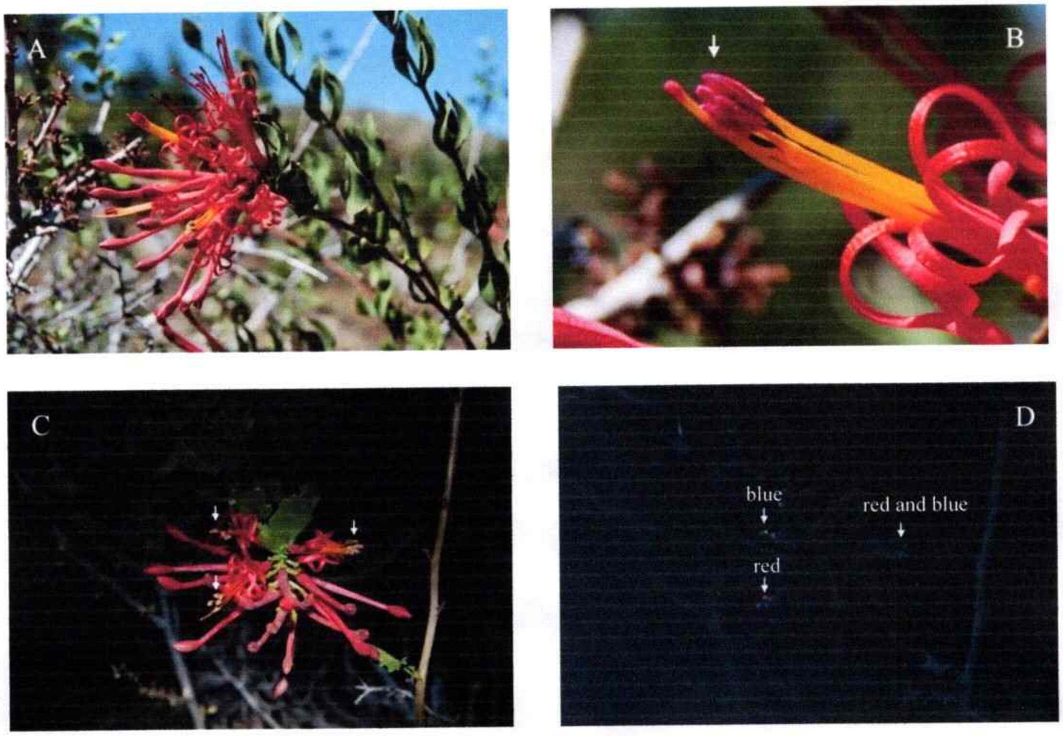

Figure 1. The mistletoe *Tristerix verticillatus.* (A) an intact inflorescence; (B) close-up of donor flower with fluorescent powder on anthers; (C) receiving flowers with fluorescent powder under white light; (D) the same receiving flowers in C under UV light. Note the different colours of fluorescent powder on the receiving flowers in D, indicating the arrival of powder from different donor HHS.

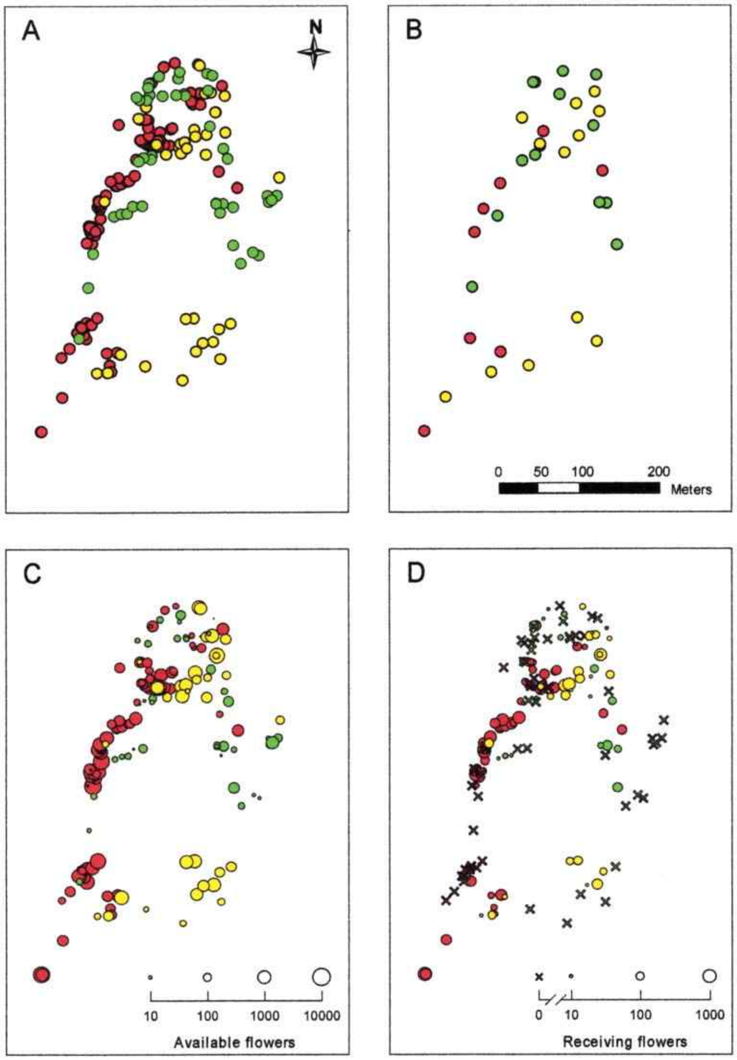

Figure 2. Infrapopulations of *T. verticillatus* available at the study site (A), donor infrapopulations of each HHS designated for the release of fluorescent powder (B), infrapopulations with their respective floral display size (C), and number of flowers of each HHS infrapopulation that received fluorescent powders (D). Each circle represent an infrapopulation and HHS are denoted by different colours: TS = red, TF = yellow, and TB = green. Data on number of flowers available (C) and number of receiving flowers (D) was $\log_{10}$ transformed to compensate for over dispersion of data; infrapopulations with no receiving flowers are shown by X's.

The flowers remained exposed to the complete assemblage of pollinators until the following night, when all opened flowers of all inflorescences of all infrapopulations of hemiparasites present in the study area were examined (receiving infrapopulations) by scanning them with a portable ultraviolet lamp (Figs. 1C and 1D; an average of 28 man-hours were spent each night tracking the arrival of fluorescent powders to each open hemiparasite flower in the study area). The code of the infrapopulation (HHS and number) and the number of flowers that received fluorescent powders on their reproductive parts were recorded. During each nightly examination, donor and receiving flowers with fluorescent powders were removed, after which a new replicate experiment was set-up. Each night, fluorescent powder was applied to one donor infrapopulation of each HHS using a different coloured dye for each HHS (thus enabling the tracking of the donor HHS in the receiving infrapopulations). This procedure was repeated in 12 separate nights; thus, a total of 36 donor infrapopulation replicates were set-up, and the movement of the fluorescent powder was tracked on the flowers of all potential receiving infrapopulations within the study area (176 infrapopulations). At the end of the same year of the pollen movement experiment (2009 fruiting season), fruit production on all the infrapopulations used for this study was also monitored.

Data analysis

GIS spatial analyses were performed using various tools of the ArcView 3.2 (ESRI 1992) software: *Nearest Neighbour Analysis* to analyse the spatial distribution of *T. verticillatus* infrapopulations in the study area (Z value: Clark & Evans 1954), *Home Range* to calculate the dispersal area of fluorescent powders, and *Point Distance* to obtain the dispersal distances of fluorescent powders from each donor infrapopulation.

Fluorescent powder movement data and fruit production was analysed by parametric ANOVA when all parametric assumptions were met; otherwise, non-parametric ANOVA on ranks (Kruskal-Wallis ANOVA) was used (Siegel & Castellan 1988; Sokal & Rohlf 1995). Floral display size and spatial distribution data were compared between the three HHS using one way ANOVA, and the number of infrapopulations that received fluorescent powders was compared using two way ANOVA with donor and receiving HHS as factors; all these statistical tests were performed with SigmaPlot 11 (Systat Software 2008). Spatial autocorrelation analysis on pollen arrival was performed with the use of correlograms and Moran's I computation; data was arcsinh transformed (see Fowler et al. 1998) and pair wise comparisons were calculated for eight distance classes (following Sturge's rule, Legendre & Legendre 1998), and a Bonferroni correction was performed with α=0.007 to determine the statistical significance of Moran's I values (Legendre & Legendre 1998); this analysis was performed in the PAST v2.16 (Hammer et al. 2001).

Additionally, since the probability of a flower receiving fluorescent powder depends on the foraging behaviour of pollinators, which in turn may be affected by factors such as floral display size (pollinators respond to resource availability) and density of floral patches (pollinators respond to resource aggregation) (Ohashi & Yahara 2001; Caraballo-Ortiz et al. 2011), a logit regression model was used to estimate the effect of both qualitative (HHS of the receiving infrapopulation as well as HHS of its nearest flowering infrapopulation) and quantitative (floral display size and nearest neighbour distance of the receiving infrapopulation) variables on the probability of reception of fluorescent dyes by any of the infrapopulations present in the study area; this regression analysis was performed with STATISTICA 8 (StatSoft 2007).

RESULTS

Floral display size and spatial distribution of *T. verticillatus* in the study area

The total number of infrapopulations and the mean number of flowers per infrapopulation in the study area are shown in table 1. The number of flowers per infrapopulation differed between the three HHS (Kruskal-Wallis ANOVA: $H_{2,177} = 46.604$; $p < 0.001$); TS and TF had more flowers than TB, but no differences in floral display size were found between TS and TF infrapopulations. The mean distances to the nearest neighbour are shown in table 1.

Table 1. Variables assessed for each hemiparasite-host system (mean ± SE). Comparisons were performed using Kruskal-Wallis ANOVA on ranks; different letters denote significant differences between HHS at $P<0.05$ using *a posteriori* comparison tests.

Variable measured	*Tristerix verticillatus* infecting three hosts		
	S. montanus	*F. imbricata*	*B. montana*
Number of infrapopulations	96	36	44
Number of flowers per infrapopulation	684 ± 113 a	638 ± 123 a	72 ± 19 b
HHS of closest neighbour to each TS	82	8	6
HHS of closest neighbour to each TF	7	23	6
HHS of closest neighbour to each TB	5	2	37
Nearest neighbour distance within HHS [m]	8.3 ± 1.2 b	23.9 ± 4.5 a	16.9 ± 2.9 a
Maximum dispersal distance [m]	172.8 ± 39.2	167.4 ± 42.9	160.5 ± 42.6
Area of dispersal [ha]	0.403 ± 0.145	0.381 ± 0.204	0.684 ± 0.171
Fruit production [number of fruits/number of flowers per inflorescence]	0.246 ± 0.043 a	0.367 ± 0.054 a	0.109 ± 0.033 b

The infrapopulations of the three HHS presented an aggregated distribution (*Nearest Neighbour Analysis*, Z = -10.79, -2.99 and -5.36 for TS, TF and TB, respectively). Moreover, the degree of aggregation differed between the three HHS (Kruskal-Wallis ANOVA over the nearest neighbour distances: $H_{2,177} = 35.441$; $p < 0.001$); *a posteriori* Dunn´s tests revealed that mean nearest neighbour distance from TS was lower (hence, the degree of aggregation higher) than that from TF ($Q = 5.412$, $P < 0.05$) and TB ($Q = 3.961$, $P < 0.05$), and that nearest neighbour distance did not differ significantly between TF and TB (Table 1). Since the three HHS co-occur within the study area and TS greatly outnumbers TF and TB (Fig. 2A, Table 1), the frequency of HHS vicinity was compared between the three HHS; and results show that despite the higher abundance of TS in the study area, all HHS have more neighbours of the same HHS (X^2: 157.86, $p < 0.001$; Table 1).

Movement of fluorescent powder among *T. verticillatus* flowers

The maximum dispersal distance of fluorescent powders from each donor infrapopulation and the resulting dispersal areas did not differ between the three HHS (Kruskal-Wallis ANOVA: $H = 1.053$, $p = 0.591$; and $H = 0.739$, $p = 0.691$; Table 1). The number of infrapopulations that received fluorescent powder was affected by the host species of the receiving HHS but not by the host species of the donor HHS ($F_{2,36} = 13.517$, $p < 0.001$ and $F_{2,36} = 0.587$, $p = 0.558$, respectively; Fig. 3A). *A posteriori* pairwise comparisons showed that the number of infrapopulations that received fluorescent powder followed the order: TS > TF > TB (Holm-Sidak tests: $t_{TS\text{-}TB} = 5.186$, $p < 0.001$; $t_{TF\text{-}TB} = 2.913$, $p =$

0.004; $t_{TS\text{-}TF} = 2.274$, $p = 0.025$). Likewise, the number of flowers that received fluorescent powder was dependent on the host species of the receiving HHS but was independent of the host species of the donor HHS ($F_{2,\,36} = 14.772$, $p < 0.001$ and $F_{2,36} = 0.737$, $p = 0.481$, respectively; Fig. 3B); more flowers with fluorescent powder were found on TS and TF than on TB (Holm-Sidak tests: $t_{TS\text{-}TB} = 5.360$, $p < 0.001$; $t_{TF\text{-}TB} = 3.462$, $p < 0.001$; $t_{TS\text{-}TF} = 1.898$, $p = 0.061$; Fig. 3B).

Overall reception (independent of donor and receiving HHS) of fluorescent powder in each infrapopulation was proportional to its own floral display size (Spearman correlation: $r_S = 0.548$; $p < 0.001$); moreover, the number of infrapopulations that received fluorescent powders decreased as the distance between the donor and receiving infrapopulations increased (Spearman correlation $r_{TS} = -0.717$, $p = 0.019$; $r_{TF} = -0.828$, $p<0.01$; $r_{TF} = -0.847$, $p<0.01$; Fig. 4A). However, the computation of Moran's I and its respective correlogram with eight distance lags indicated the absence of spatial autocorrelation on pollen analogue arrival (Fig. 4B) which was also supported by the results of the logit regression model performed to weigh out the effect of quantitative (floral display size and infrapopulation proximity) and qualitative (HHS of receptor and HHS of receptor's closest neighbour) variables on the probability of dye reception showed that none of the quantitative variables tested significantly predicted the arrival of fluorescent powder to any given infrapopulation (Wald $\text{statistic}_{\text{Floral display size}}$: 3.54, $p = 0.06$; Wald statistic$_{\text{Infrapopulation proximity}}$: 2.86, p=0.09). Moreover, among the qualitative variables, only host species of the receiving infrapopulation but not the HHS of the nearest infrapopulation significantly predicted the arrival of fluorescent powder to any given infrapopulation (Wald statistic $_{\text{Receiving HHS}}$: 11.11, p<0.01; Wald statistic$_{\text{Nearest neighbour HHS}}$: 0.86, p = 0.65; Wald statistic $_{\text{Receiving HHS*Nearest neighbour HHS}}$: 6.82, p = 0.14). The model was able to correctly predict the arrival of powder in 90.5% of cases.

Finally, ca. 25% of the developed flowers produced fruits; there was a significant host effect on fruit production ($H_{2,176} = 17.828$, $p < 0.001$), and the success of fruit production (number of fruits/number of flowers per inflorescence) did not differ between TS and TF, but both produced more fruits than TB (Table 1).

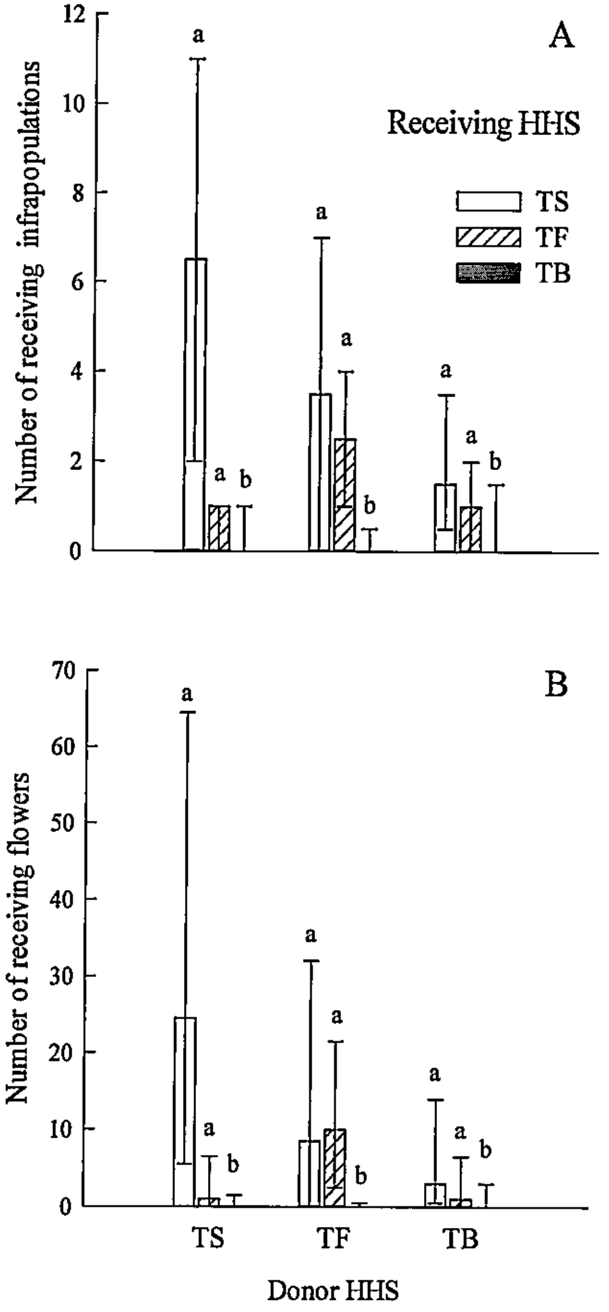

Figure 3. Number of infrapopulations (a) and flowers (b) of different HHS that received fluorescent powders from different donor HHS. Median and interquartile ranges are shown and different letters within each HHS denote significant differences at $P<0.05$ using the *a posteriori* comparison tests.

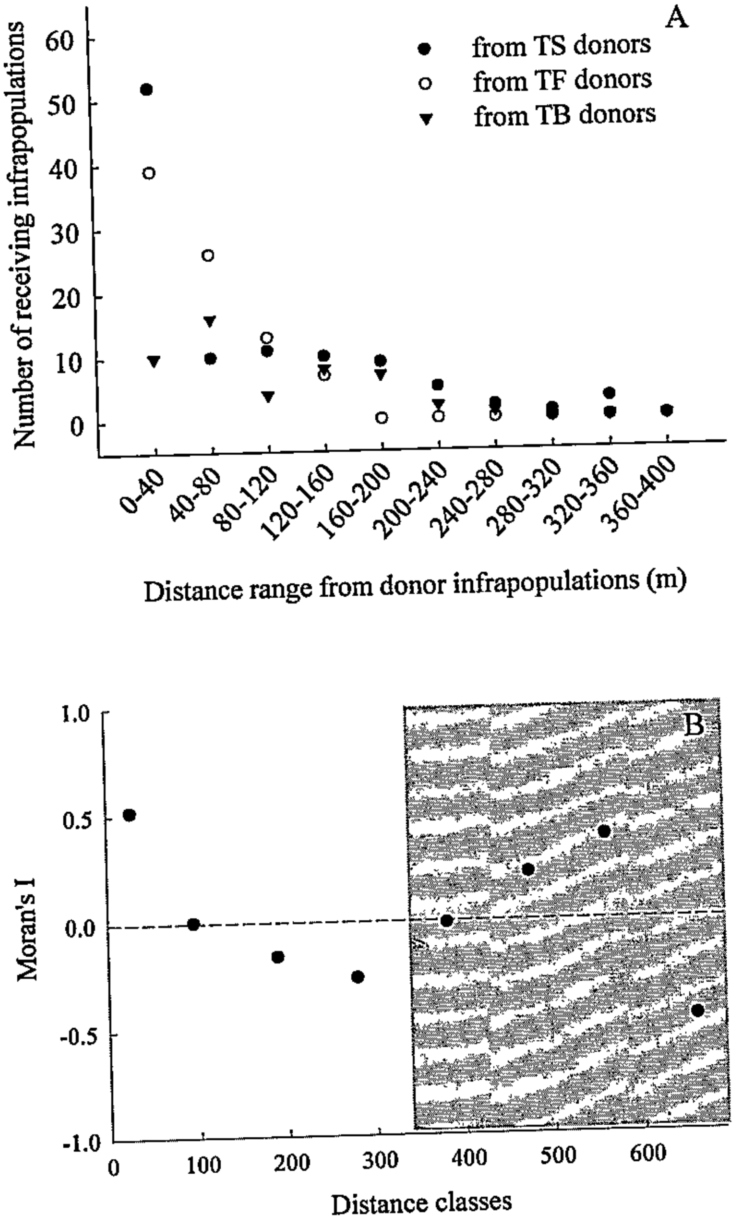

Figure 4. Pollen analogue arrival with respect to donor infrapopulations. A) Number of infrapopulations which received fluorescent powders as a function of the distance between donor and receiving infrapopulations, for different donor HHS, B) Moran's I correlogram depicting the absence of spatial autocorrelation on pollen reception.

DISCUSSION

We hypothesised that individuals of *T. verticillatus* on *S. montanus* will export and receive more pollen than the other two hemiparasite-host systems. Results show that although the three HHS did not differ in terms of fluorescent powder exportation nor in donor effect on the number of infrapopulations and number of flowers that received fluorescent powder, a higher movement of fluorescent powder towards the TS system occurred (significant receiving HHS effect on the number of infrapopulations and number of flowers that received fluorescent powder). Furthermore, no differences were observed in the area and maximum distance of dispersal between the three HHS, indicating that the dispersal range of fluorescent powders from donors of the TS system was comparable with that of TF and TB. In summary, the TS system did receive more but it did not export more pollen analogue than TF and TB; therefore, our hypothesis was only partially confirmed.

At YLNS, the most infected and intensely parasitised host is *S. montanus* (Lemaitre et al. 2011) and TS infrapopulations present a higher degree of aggregation and are more abundant than TF and TB. However, even though the three HHS coexist sympatrically and TS outnumbers TF and TB, the closest neighbour of most infrapopulations is another infrapopulation of the same HHS (this study); hence, pollinators, which are expected to optimise their flying costs when foraging, would travel shorter distances to the next floral patch with a higher probability that the next receiving infrapopulation will also be of the same host species. Contrastingly, the quantitative examination of the problem through a logit regression analysis showed that host but not floral display size nor level of aggregation of the receiving infrapopulations was a significant predictor of powder arrival (with more than 90% confidence). The pattern of pollen analogue movement found is consistent with the tendency revealed in our previous study where the TS system presented more visits by insect pollinators to its associated *T. verticillatus* flowers, a fact mainly attributed to volatile chemistry differences affecting the attraction of certain types of pollinators (Troncoso et al. 2010). Floral display size and plant density can in no way be dismissed as important cues for pollinator arrival and foraging behaviour (Grindeland et al. 2005; Nattero et al. 2011), but our results highlight the importance of host-plant species over floral display size and plant density for the arrival of fluorescent powder, which under our perspective is mainly led by HHS chemistry (both volatile chemistry and nectar production). It should be mentioned that other factors potentially affecting pollen arrival such as flower size and colour, and pollen production were not incorporated in the model because they were found not to vary between HHS (results not shown); on the other hand, nectar production, which indeed varies between HHS (TS produces more nectar than TF and TB, results not shown), was not performed because its value prior to visitations cannot be determined since flowers must be destroyed for its measurement; hence the pattern of nectar production is considered within the set of intrinsic traits of each HHS, such as volatile chemistry.

Follow-up monitoring of the hemiparasite individuals on the study area revealed that there were no differences in fruit production (number of fruits/number of flowers per inflorescence) between TS and TF, but both produced more fruits than TB (Table 1). All species of the genus *Tristerix* produce one-seeded berries; hence, they are unlikely to be pollen-limited (Burd et al. 2009). Instead, the comparable production of fruits between TS and TF, despite the higher levels of pollen movement towards TS and the higher level of infection of *S. montanus* (Lemaitre et al. 2011), suggests that fruit production in this species may be resource limited.

Movement of fluorescent powder has been considered a reliable proxy for pollen movement (Kearns & Inouye 1993; Adler & Irwin 2006), and has shown consistent results when compared with effective pollen dispersal (Van Rossum et al. 2011). We have shown a prevalent fluorescent powder movement to the TS system, and provided a proxy of pollen reaching the stigma of a flower different from the source flower (potential pollen movement); whether such pollen effectively led to the production of seed (realised pollen movement) is beyond the reach of this method. We are aware that this latter evaluation can only be accomplished through paternity analysis using microsatellite markers to identify the pollen donors among all potential parents in the population (Van Rossum et al. 2011; Matsuki, Tomita & Isagi 2011). However, no microsatellite markers have been published for this species nor for any other Loranthaceae species; hence, although extremely desirable, such paternity analyses still remain our long-term goal. Nonetheless, our study provides the first indication of non-panmictic host-mediated pollen movement within the population of *Tristerix verticillatus*. To date, although ecological speciation has been hypothesised for the genus *Tristerix* (Amico et al. 2007), host-mediated emergence of genetic races in mistletoes has been reported only in allopatric populations of *Arceuthobium americanum* (Viscaceae) (Jerome & Ford 2002).

ACKNOWLEDGEMENTS

We are indebted to FONDECYT (grant 1080248 to HMN) and to the International Foundation for Science (grant 4356-1 to AJT) for financial support. We thank I. Municipalidad de Lo Barnechea for a permit to perform the studies at the Yerba Loca Nature Sanctuary.

REFERENCES

Adler L. S. (2000) Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *Am.Nat.* **156**, 92-99.

Adler L. S. & Irwin R. E. (2006) Comparison of pollen transfer dynamics by multiple floral visitors: Experiments with pollen and fluorescent dye. *Ann. Bot.* **97**, 141-150.

Amico G. C., Vidal-Russell R. & Nickrent D. L. (2007) Phylogenetic relationships and ecological speciation in the mistletoe *Tristerix* (Loranthaceae): the influence of pollinators, dispersers, and hosts. *Am. J. Bot.* **94**, 558–567.

Bailey J. K., Wooley S. C., Lindroth R. L. & Whitham T. G. (2006) Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Let.* **9**, 78-85.

Bukovinszky T., van Veen F. J. F., Jongema Y. & Dicke, M. (2008) Direct and indirect effects of resource quality on food web structure. *Science* **319**, 804-807.

Burd M., Ashman T. L., Campbell D. R., Dudash M. R., Johnston M. O., Knight T. M., Mazer S. J., Mitchell R. J., Steets J. A. & Vamosi J.C. (2009) Ovule number per flower in a world of unpredictable pollination. *Am. J. Bot.* **96**, 1159-1167.

Caraballo-Ortiz M. A., Santiago-Valentin E. & Carlo, T. A. (2011) Flower number and distance to neighbours affect the fecundity of *Goetzea elegans* (Solanaceae). *J. Trop. Ecol.* **27**, 521-528.

Clark P. J. & Evans F. C. (1954) Distance to nearest neighbour as a measure of spatial relationships in populations. *Ecology* **35**, 445-453.

Dobson, H. E. M. (2006) Relationship between floral fragrance composition and type of pollinator. In: *Biology of Floral Scents* (eds N. Dudareva & E. Pichersky) pp 147-198. CRC Press Taylor & Francis Group, Boca Raton.

ESRI (1992) *ArcView GIS version 3.2.* Redlands, California.

Fowler J., Cohen L. & Jarvis P. (1998) Practical statistics for field biology. John Wiley & Sons, Chichester.

Gajardo R. (1994) *La vegetación natural de Chile: Clasificación y distribución geográfica.* Editorial Universitaria, Santiago.

Grindeland J. M., Sletvold N. & Ims R. A. (2005) Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Funct. Ecol.* **19**, 383-390.

Hammer Ø., Harper D. A. T. & Ryan P. D. (2001) PAST: Paleontological Statistics software package for education and data analysis. *Paleontologia Electronica* **4**, 9.

Huyse T., Poulin R. & Théron A. (2005) Speciation in parasites: a population genetics approach. *Trends Parasitol.* **21**, 469-475.

Jerome C. A. & Ford B. A. (2002) The discovery of three genetic races of the dwarf mistletoe *Arceuthobium americanum* (Viscaceae) provides insight into the evolution of parasitic angiosperms. *Mol. Ecol.* **11**, 387-405.

Kearns C. A. & Inouye D. W. (1993) *Techniques for pollination biologists.* University Press of Colorado, Colorado.

Kessler D., Gase K. & Baldwin I. T. (2008) Field experiments with transformed plants reveal the sense of floral scents. *Science* **321**, 1200-1202.

Knudsen J. T., Tollsten L., Groth I., Bergström G. & Raguso R. A. (2004) Trends in floral scent chemistry in pollination syndromes: floral scent composition in humming-bird pollinated taxa. *Bot. J. Linn. Soc.* **146**, 191-199.

Kuijt J. (1988) Systematic Botany Monographs. Revision of *Tristerix* (Loranthaceae). The American Society of Plant Taxonomists. 19, 61 pp.

Legendre P. & Legendre L. (1998) Numerical ecology. 2nd English edition. Elsevier, Amsterdam

Lemaitre A., Troncoso A. J. & Niemeyer H. M. (2011) Host preference of a temperate mistletoe: disproportional infection on three co-occurring host species influenced by differential success. *Aust. Ecol.* doi:10.1111/j.1442-9993.2011.02281.x.

Matsuki Y., Tomita M. & Isagi Y. (2011) Pollination efficiencies of insects visiting *Magnolia obovata*, as determined by single-pollen genotyping. In: *Single-Pollen Genotyping*. (eds Y. Isagi & Y. Suyama) pp 17-32. Springer, Tokyo.

Nattero J., Malerba R., Medel R. & Cocucci A. (2011) Factors affecting pollinator movement and plant fitness in a specialized pollination system. *Plant Syst. Evol.* **296**, 77-85.

Ohashi, K., & Yahara, T. (2001) Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: *Cognitive ecology of pollination*. (eds L. Chittka & J. D. Thomson) pp 274-296. Cambridge University Press, New York.

Poelman E. H., van Dam N. M., van Loon J. J. A., Vet L. E. M. & Dicke M. (2009) Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. *Ecology* **90**, 1863-1877.

Power M. E. (1992) Top-Down and Bottom-Up Forces in Food Webs: Do Plants Have Primacy. Ecology **73,**733

Raguso R. A. (2008) Start making scents: the challenge of integrating chemistry into pollination ecology. *Entomol. Exp. Appl.* **128**, 196–207.

Siegel S. & Castellan Jr. N. J. (1988) *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, London.

Sokal R. R. & Rohlf F. J. (1995) *Biometry*, 3rd ed. W. H. Freeman and Company, New York.

StatSoft Inc. (2007) *STATISTICA (data analysis software system), version 8.0.* www.statsoft.com.

Systat Software. (2008) *SigmaPlot version 11*. San Jose, California.

Troncoso A. J., Cabezas N. J., Faúndez E., Urzúa A. & Niemeyer H. M. (2010) Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. *Oecologia* **162**, 413-425.

Van Rossum F., Stiers I., Van Geert A., Triest L. & Hardy O. J. (2011) Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. *Oecologia* **165**, 663-674.

Waser N. M. & Price M. V. (1982) A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **63**, 1168-1172.

DISCUSION GENERAL

Las plantas parásitas generalistas pueden mostrar preferencia local por unos hospederos por sobre otros dentro su ámbito de distribución, que en general se ha visto relacionada con la abundancia local de hospederos (Norton & Carpenter 1998). No obstante, *T. verticillatus* ha demostrado ser capaz de parasitar una gama de hospederos y comportarse como un parásito que demuestra preferencia por *S. montanus*, ya que este hospedero es infectado con mayor incidencia e intensidad, y además independientemente de su abundancia y apariencia local (Capítulo 1). Entre los posibles mecanismos que expliquen el patrón de infección (*alias* forrajeo) observado, se examinaron: i) la distribución espacial, ii) abundancia y apariencia, y iii) diferencias en el éxito de establecimiento.

Fue este último mecanismo el que encontró mejor soporte gracias a los experimentos de inoculación cruzada, cuyos resultados evidenciaron que existe un efecto de hospedero de procedencia que determina el éxito de establecimiento en *T. verticillatus*, fenómeno que también ha sido reportado para otras especies de plantas hemiparásitas aéreas (Clay et al. 1985, Rödl & Ward 2002, Lara et al. 2009). El mecanismo subyacente al reconocimiento del hospedero durante el desarrollo haustorial de *T. verticillatus* no ha sido investigado aún; no obstante, el rol de señales químicas en el crecimiento dirigido hacia la búsqueda y selección de hospedero por plantas parásitas ha sido ya reportado (Runyon et al. 2006).

Los individuos de *T. verticillatus* ya establecidos, reciben una influencia ascendente de sus hospederos que determina la intensidad de las interacciones que mantiene *T. verticillatus* con sus polinizadores y herbívoros, como se evidencia en el capítulo 2. Normas de interacción ocurren en las interacciones con polinizadores y también con herbívoros (Cap. 2, fig. 4). Podemos encontrar paralelismos entre estos hallazgos con los efectos ascendentes documentados en sistemas tritróficos planta hospedero – herbívoro – mutualista (Abbot et al. 2008, Cushman 1991, Reithel & Billick 2006, Mooney & Agrawal 2008), y planta hospedero – herbívoro – antagonista (i.e. Johnson 2008, Schädler et al. 2010, Gols et al 2008 a,b, Gols & Harvey 2009).

En el caso de la herbivoría, la intensidad de interacción no difiere entre HHS cuando se compara a nivel *per capita*, pero sí a nivel poblacional (Cap. 2, fig. 4); por tanto, la intensidad de interacción con herbívoros depende directamente de las abundancias relativas de cada sistema hemiparásito-hospedero en el sitio de estudio. Efectos similares de la abundancia relativa de especies sobre la estructura de redes de interacción ya han sido reportadas por Vásquez (2007, 2009 a,b). En el caso particular de *T. verticillatus*, la relación hemiparásito-hospedero durante el establecimiento (Cap. 1, Tabla 1) ha sido el mecanismo más probable que da cuenta de la mayor abundancia del sistema TS.

En cuanto a la polinización, la mediación de los hospederos sobre la interacción entre *T. verticillatus* y sus polinizadores es un fenómeno que ocurre a nivel *per capita* y se acentúa a nivel poblacional (Cap. 2, fig. 4). Los compuestos volátiles emitidos (Troncoso et al. 2010) y la producción de néctar (Volumen producido = media ± SD, µl/24h; TS=21.88 ± 2.23, TF=14.27 ± 4.88 y TB=18.88 ± 4.55; $F_{2,42}$=7.51, *P*=0.002; com. pers.) pueden considerarse como las causas más probables del evidente sesgo hacia el mayor uso de TS por parte del ensamble de polinizadores. Por otro lado, a pesar de que la fuerza de interacción de los polinizadores demostró tener un efecto leve pero significativamente positivo sobre la producción de frutos, una métrica de adecuación biológica femenina para *T. verticillatus*, ésta no difiere entre los sistemas hemiparásito-hospedero y se sugiere que puede estar limitada por recursos, aspecto del sistema que merece ser explorado en profundidad.

La dominancia del sistema *Tristerix verticillatus/Schinus montanus* a nivel intrapoblacional y en términos de fuerza de interacción con polinizadores también se ve reflejada en el patrón de dispersión de los análogos de polen dentro la población de *T. verticillatus,* fenómeno que demostró ser independiente de la oferta floral y grado de agregamiento (Cap. 3, Tabla 1 y fig. 4B). Los resultados sobre movimiento potencial de polen indican la llegada sesgada de polen hacia el sistema *Tristerix verticillatus/Schinus montanus*; la corroboración final de este fenómeno deberá realizarse a través estudios moleculares de análisis de paternidad (por ejemplo, Van Rossum et al. 2011), los cuales todavía están fuera del alcance de la presente tesis por motivos inherentes a la optimización de protocolos para esta especie.

Finalmente, el conjunto de patrones encontrados en la interacción de *T. verticillatus* con sus hospederos, tales como preferencia de hospedero, efecto del hospedero sobre las interacciones del parásito, e incluso la mediación del hospedero sobre los patrones de reproducción (e.g., movimiento de polen), guardan semejanza con patrones ampliamente documentados en interacciones herbívoro-hospedero (revisado por Pennings & Callaway 1991; preferencia de hospedero: Bernays & Chapman 1994; apareamiento estructurado: Caillaud & Via 2000).

La adaptación a hospederos es un factor importante en la evolución de parásitos como poblaciones que pueden eventualmente diferenciarse en razas (Jerome & Ford 2002; Thompson 1994). No obstante, la especiación en parásitos puede también ocurrir en ausencia de especialización, como resultado de un flujo génico disminuido debido a migración limitada (aislamiento por distancia), hospederos en poblaciones parchosas o como resultado de presiones de selección divergentes impuestas por condiciones ecológicas distintas (Gerardo et al. 2004; Kuijt 1969; Lowe et al. 2002; Norton & Carpenter 1998). No se ha comprobado la ocurrencia de apareamiento estructurado para el sistema bajo estudio; no obstante, la potencialidad de ocurrencia de éste como mecanismo de especiación en este grupo de plantas ya ha sido sugerido por Amico et al. (2007), quienes proponen que la especiación dentro del género *Tristerix* en Sudamérica es el producto de su interacción con sus hospederos y polinizadores.

REFERENCIAS

Abbot P, Grinath J, Brown J, Peeden E, Erickson D, Billick I (2008) Insect herbivore stoichiometry: the relative importance of host plants and ant mutualists. Ecol Entomol 33,497–502

Adler LS (2000) Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. Am Nat 156,92-99

Adler LS (2003) Host species affects herbivory, pollination, and reproduction in experiments with parasitic *Castilleja*. Ecology 84,2083–2091

Amico GC, Vidal-Russell R, Nickrent DL (2007) Phylogenetic relationships and ecological speciation in the mistletoe *Tristerix* (Loranthaceae): the influence of pollinators, dispersers, and hosts. *Am. J. Bot.* 94,558–567

Attsat PR (1977) The insect herbivore as a predictive model in parasitic seed plant biology. Am Nat 111,579-586

Berlocher SH, Feder JL (2002) Sympatric speciation in phytophagous insects: moving beyond controversy? Ann Rev Entomol 47,773–815

Bernays EA, Chapman RF (1994) Host-plant selection by phytophagous insects. London, Chapman and Hall.

Cabezas NJ, Urzúa AM, Niemeyer HM (2009) Translocation of isoquinoline alkaloids to the hemiparasite, *Tristerix verticillatus* from its host, *Berberis montana*. Biochem Syst Ecol 35,225-227

Caillaud CM, Via S (2000) Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphid. Am Nat 156,606–621

Clay K, Dement D, Rejmanek M (1985) Experimental evidence for host races in mistletoe (*Phoradendron tomentosum*). Am. J. Bot. 72,1225-1231

Cushman JH (1991) Host-plant mediation of insect mutualisms: variable outcomes in herbivore–ant interactions. Oikos 61,138-144

Gerardo NM, Mueller UG, Price SL, Currie CR (2004) Exploiting a mutualism: parasite specialization on cultivars within the fungus-growing ant symbiosis. Proc R Soc Lond Ser B 271,1791-1798

Gols R, Raaijmakers CE, van Dam NM, Dicke M, Bukovinszky T, Harvey JA (2007) Temporal changes affect plant chemistry and tritrophic interactions. Basic Appl Ecol 8,421–433

Gols R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM & Harvey JA (2008a) Performance of generalist and specialist herbivores and their endoparasitoids differ on cultivated and wild *Brassica* populations. J Chem Ecol 34,132–143

Gols R, Wagenaar R, Bukovinszky T, van Dam NM, Dicke M et al. (2008b) Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology 89,1616–1626

Hunter MD, Price PW (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom up and top down forces in natural communities. Ecology 73,724–732

Huyse T, Poulin R, Théron A (2005) Speciation in parasites: a population genetics approach. Trends Parasitol 21,469-475

Jerome CA, Ford BA (2002) The discovery of three genetic races of the dwarf mistletoe *Arceuthobium americanum* (Viscaceae) provides insight into the evolution of parasitic angiosperms. Mol Ecol 11,387-405

Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants and predators. Ecology 89,145–154

Kuijt J (1969) The biology of parasitic plants. University of California Press, Berkeley

Kuijt J (1988) Systematic Botany Monographs. Revision of *Tristerix* (Loranthaceae). Am Soc of Plant Taxon 19,1-61

Lara C, Pérez G, Ornelas JF (2009) Provenance, guts, and fate: field and experimental evidence in a host-mistletoe-bird system. Ecoscience 16, 399-407

Lei SA (1999) Age, size and water status of *Acacia gregii* influencing the infection and reproductive success of *Phoradendron californicum.* Am Midl Nat 141,358-365

Lowe RM, Ward SA, Crozier RH (2002) The evolution of parasites from their hosts: intra- and interspecific parasitism and Emery's rule. Proc R Soc Lond Ser B 269,1301-1305

Lymbery AJ (1989) Host specificity, host range and host preference. Parasitol Today 5,298

Medel R, Vergara E, Silva A, Kalin-Arroyo M (2004) Effects of vector behavior and host resistance on mistletoe aggregation. Ecology 85,120-126

Mooney KA, Agrawal AA (2008) Plant genotype shapes ant–aphid interactions: implications for community structure and indirect plant defense. Am Nat 171,E195–E205

Norton DA, Carpenter MA (1998) Mistletoes as parasites: host specificity and speciation. Trends Ecol Evol 13,101-105

Pennings SC, Callaway RM (2002) Parasitic plants: parallels and contrasts with herbivores. Oecologia 131,479-489

Poulin R (2007) Evolutionary Biology of Parasites: From Individuals to Communities. New Jersey: Princeton University Press.

Poulin R, Mouillot D (2003) Parasite specialization from a phylogenetic perspective: a new index of host specificity. Parasitology 126,473-480

Power ME (1992) Top-Down and Bottom-Up Forces in Food Webs: Do Plants Have Primacy. Ecology 73,733-746

Reithel JS, Billick I (2006) Bottom-up mediation of an ant–membracid mutualism: effects from different host plants. Evol Ecol 20,27–38

Rödl T, Ward D (2002) Host recognition in a desert mistletoe: early stages of development are influenced by substrate and host origin. Funct Ecol 16,128-134

Roughgarden J, Pennington T, Alexander S (1994) Dynamics of the rocky intertidal zone with remarks on generalizations in ecology. Phil Trans R Soc Lond Ser B 343,79–85

Runyon JB, Mescher MC, De Moraes CM (2006) Volatile chemical cues guide host location and host selection by parasitic plants. Science 313,1964-1967

Schädler M, Brandl R, Kempel A (2010) Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. Entomol Exp et Appl 135,162–169

Strauss SY (1997) Floral characters link herbivores, pollinators, and plant fitness. Ecology 78,1640–1645

Thompson JN (1994) The Coevolutionary Process. Chicago: Chicago University Press

Troncoso AJ, Cabezas NJ, Faúndez EH, Urzúa A, Niemeyer HM (2010) Host-mediated volatile polymorphism in a parasitic plant influences its attractiveness to pollinators. Oecologia 162,413-425

van Ommeren RJ, Whitham TG (2002) Changes in interactions between juniper and mistletoe mediated by shared avian frugivores: parasitism to potential mutualism. Oecologia 130,281-288 van Rossum F, Stiers I, Van Geert A, Triest L, Hardy OJ (2011) Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. Oecologia 165,663-674

Vázquez DP, Blüthgen N,Cagnolo L, Chacoff NP (2009a) Uniting pattern and process in plant-animal mutualistic networks: a review. Ann Bot 103,1445-1457

Vázquez DP, Chacoff N, Cagnolo L. (2009b) Evaluating multiple determinants of the structure of mutualistic networks. Ecology 90, 2039-2046

Vázquez DP, Melián CJ, Williams NM, Blüthgen N, Krasnov BR, Poulin R (2007) Species abundance and asymmetric interaction strength in ecological networks. Oikos 116,1120-1127