

Generalist birds govern the seed dispersal of a parasitic plant with strong recruitment constraints

Ana Mellado · Regino Zamora

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Abstract Mistletoes constitute instructive study cases with which to address the role of generalist consumers in the study of plant–animal interactions. Their ranges of safe sites for recruitment are among the most restricted of any plant; therefore, frugivores specializing in mistletoe have been considered almost indispensable for the seed dispersal of these parasitic plants. However, the absence of such specialists in numerous regions inhabited by many mistletoe species raises the question of whether unspecialized vectors may successfully disperse mistletoe seeds to narrowly defined safe sites. Using the European mistletoe *Viscum album* subsp. *austriacum* as a study case, we recorded a broad range of 11 bird species that disperse mistletoe seeds. For these species, we studied the mistletoe-visitation rate and feeding behavior to estimate the quantity component of dispersal effectiveness, and the post-foraging microhabitat use, seed handling, and recruitment probabilities of different microhabitats as a measure of the quality component of effectiveness. Both endozoochory and ectozoochory are valid dispersal mechanisms, as the seeds do not need to be ingested to germinate, increasing seed-dispersal versatility. Thrushes were the most effective dispersers, although they were rather inefficient, whereas small birds (both frugivores and non-frugivores) offered low-quantity but high-quality services for depositing seeds directly upon safe sites. As birds behave similarly on parasitized and non-parasitized hosts, and vectors have broad home ranges, reinfection

within patches and the colonization of new patches are ensured by an ample assemblage of generalist birds. Thus, a parasitic plant requiring precision in seed dispersal can rely on unspecialized dispersers.

Keywords Ectozoochory · Endozoochory · Generalist system · Mistletoe · Viscaceae

Introduction

Most theoretical and empirical studies on plant–animal interactions have traditionally outlined the role of specialization as a prominent feature of interactive systems. However, an increasing amount of scientific evidence strongly supports the idea that generalization is more frequent than hitherto thought, with most plants interacting with multiple types of pollinators, seed dispersers, and herbivores (Herrera 1988; Waser et al. 1996; Gómez and Zamora 2000; Olesen and Jordano 2002; Bascompte and Jordano 2007). Mistletoes are widely distributed worldwide as common colonizers of tree canopies and constitute excellent models with which to examine the issue of generalization versus specialization. Their parasitic nature limits them to a narrowly defined range of safe sites for seed recruitment, i.e., particular branch diameters of specific host species (Reid 1989; Van 1993; Sargent 1995; Norton and de Lange 1999; Aukema and Martínez de Río 2002; Arruda et al. 2006). Such strong specificity suggests that mistletoes must depend on highly precise dispersal mechanisms to guide their seeds to safe sites. In this regard, many frugivore species reportedly have close mutualist ties with mistletoes, exchanging effective seed-dispersal services for food rewards (Reid 1990; Wenny 2001; Amico and Aizen 2000; Carlo and Aukema 2005), reflecting a likely coevolution

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A. Mellado (✉) · R. Zamora
Department of Ecology, Terrestrial Ecology Research Group,
University of Granada, Av. Fuentenueva s/n, 18071 Granada,
Spain
e-mail: anamegar@ugr.es

(Reid 1991; Aukema and Martínez del Río 2002). However, this tight dependence of mistletoes on specialized vectors has been increasingly questioned, since many dietary generalists have been described as frequent mistletoe seed dispersers (e.g., Restrepo 1987; Reid 1991; Hawksworth and Wiens 1996; Zuber 2004; Rawsthorne et al. 2011; Arruda et al. 2012). In fact, several mistletoe species inhabiting large territories where adept vectors are completely absent depend exclusively on such dietary generalists, e.g., most mistletoe species dwelling in the Northern Hemisphere, on many ocean islands, and in several regions of the Southern Hemisphere (Reid 1991; Watson and Rawsthorne 2013).

Mistletoes relying on specialist vectors—all bird species (Restrepo et al. 2002)—may take advantage of the exclusive, direct seed dispersal of a few legitimate dispersers (Davidar 1983; Reid 1989; Martínez del Río et al. 1995; Sargent 1995; Larson 1996). These birds, with their extremely restricted diet, ensure that mistletoe fruits are consumed, as they subsist almost completely on them (Reid 1991; Restrepo et al. 2002; Watson 2004); they also guarantee high-quality dispersal services due to special anatomical and behavioral adaptations (Walsberg 1975; Richardson and Wooller 1988) which enhance seed germination or seedling establishment after seeds pass through the digestive tract (e.g., Martínez del Río and Restrepo 1993; Murphy et al. 1993) and encourage seedling recruitment by depositing seeds on safe sites (e.g., Reid 1989; Wenny 2001). On the other hand, mistletoes that depend on generalist vectors may risk the uncertainty that fruits may not be consumed or seeds may not arrive at suitable habitats (Reid 1989; Larson 1996; Montaña-Centellas 2012; Watson 2012). Despite the frequency of these parasitic plants, the way in which mistletoes that rely exclusively on generalists achieve their dissemination remains unknown.

In this study, we investigate dispersal by a group of birds that feed on the mistletoe *Viscum album* subsp. *austriacum*. This parasitic plant is widely distributed across Europe, where no mistletoe specialists exist and, therefore, where seed dispersal depends exclusively on generalists (Snow and Snow 1984, 1988; Zuber 2004; Watson and Rawsthorne 2013). We explored how this bird assemblage affects the demography of mistletoe by analyzing relative dispersal effectiveness [i.e., the contribution of a seed disperser to mistletoe fitness, sensu Schupp (1993)] and efficiency (i.e., the probability that a mistletoe seed will arrive at a safe site). For each mistletoe visitor, we studied the visitation rate and feeding behavior to estimate the “quantity” component of the effectiveness; also we examined post-foraging microhabitat use and the consequent seed shadows to assess the “quality” component of effectiveness. Concurrently, through an experimental approach, we quantified recruitment probabilities of different microhabitats within suitable hosts. We also noted whether these birds were capable of depositing

seeds at appropriate safe sites beyond the mother plant, acting as colonizers of new infection foci. With this approach, we seek to understand how a parasitic plant requiring precision in seed dispersal can rely on an imprecise, unspecialized dispersal assemblage for reinfection (establishing “infection centers”), as well as for transmission to suitable new hosts.

Materials and methods

Study system and site

Viscum album subsp. *austriacum* (Wiesb.) Vollman, a mistletoe species native to most regions of Europe, specializes in parasitizing conifers, mainly *Pinus* species across its distribution range. The study site, the Sierra de Baza (2°51'48"W, 37°22'57"N), represents the southernmost limit of its geographical distribution. This is a mountainous natural reserve of southeastern Spain, which shows a sharp altitudinal gradient of 850–2270 m. The climate is typically Mediterranean, with hot, dry summers (June–September), cold winters (December–March), and rainy autumns and springs. Pine is the dominant forest tree, with Austrian pine (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.) being major hosts for *V. album* subsp. *austriacum* at this site. Other pines, Aleppo (*Pinus halepensis* Mill.) and maritime (*Pinus pinaster* Ait.), as well as non-conifer tree species, holm oaks (*Quercus ilex* L.) and *Acer opalus* L. subsp. *granatense* Boiss., are also abundant. As a bird-dispersed plant, *V. album* subsp. *austriacum* produces copious crops of whitish fleshy fruits that ripen at the beginning of September and remain available for frugivores until early March. This mistletoe shares an assemblage of seed dispersers with a diverse community of fleshy fruited shrubs formed chiefly by *Berberis hispanica* subsp. *hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq., *Lonicera arboorea* Boiss., *Juniperus oxycedrus* L., *Juniperus communis* L., *Prunus ramburii* Boiss., and *Rosa* sp.

Mistletoe recruitment and seed-deposition sites

We studied the natural seed-deposition pattern of *V. album* subsp. *austriacum* on pine branches after a seed-dispersal season (beginning of March 2010). Mistletoe seed rain was measured on 41 branches of 41 parasitized *P. nigra* trees of similar parasitic loads (hosting from five to ten large-sized fruiting mistletoes). One of the accessible branches per tree was randomly selected for study. Each branch was divided into four positions according to branch thickness: the thickest (>2.5 cm) and nearest position to the trunk (basal position); the middle position of the branch (2.49–1 cm) (middle position); and two apical positions (0.99–0.2 cm), one uncovered (pine twigs 2–3 years old) (apical uncovered),

and the other covered by pine needles (the most recent pine growth) (apical covered). Branch diameter was measured with a precision caliper. For each branch position we quantified the number of mistletoe seeds, considering losses due to seed predation as predators feed mostly on the embryo (Grazi and Urech 2000 in Zuber 2004), leaving easily detectable scrapes on the branch.

Also, we estimated the quality of each branch position for mistletoe recruitment by calculating recruitment probabilities at these sites. To do so, we performed a sowing experiment under field conditions. Ten *P. nigra* trees, alike in age (40 year), size (diameter at breast height, 10.27 ± 1.38 cm), architecture, and ecological environment, were chosen in Sierra de Baza. Three branches per tree were randomly assigned and divided into four positions, following the same criteria as for seed counts. A total of 900 seeds were placed onto the 30 branches: ten seeds in basal positions (>2.5 cm), ten in middle positions (2.49–1 cm), five in uncovered apical positions (0.99–0.2 cm), and five in needle-covered apical positions. Fruits collected in Sierra de Baza during early March 2010 from 15 source plants growing on different host trees were pooled and randomized. Fruit exocarps were removed to permit seed germination (Ladley and Kelly 1996) and seeds were rapidly and carefully placed on branches, leaving 1.5- to 2-cm spaces between seeds. After 15 months (considered sufficient time for seeds to germinate and to become established), we quantified the number of seedlings at each branch position. Then we estimated recruitment probabilities of each site as the number of seedlings counted at the end of the experiment relative to the initially placed seeds.

Identification of mistletoe seed dispersers and seed-deposition patterns

We investigated the behavioral patterns of all members of the frugivore bird assemblage inhabiting the study site, as well as their linkage to mistletoe dispersal. For this, we recorded number of bird visits to parasitized *P. nigra* and described their feeding and post-foraging behavior on different host structures. Additional bird watching in non-parasitized trees served to compare avian behavioral patterns when mistletoes were present or absent. For two seed-dispersal seasons, from September to February of 2 consecutive years (2009–2010 and 2010–2011), birds were observed by direct observations as well as videotaping. Direct observations, from 0700 to 1200 hours at four localities of the study site, were randomly conducted over the sampling period from hiding places at a minimum distance of 15 m from focal trees (using binoculars when needed for bird identification). For videotaping, high-resolution video cameras were placed directly in the field, from 0700 to 1200 hours. Recordings were made on 48 parasitized *P.*

nigra trees from distances of 5–15 m, enabling bird identifications and accurate descriptions of feeding behavior.

In the study of avian feeding behavior, each time a bird made contact with the mistletoe was considered a “visit.” During each visit, the visitor was identified to the species level, the activity (feeding or perching), fruit-removal rate (number of fruits swallowed per minute) and visit duration were recorded. We disregarded incomplete observations when estimating visit durations, i.e., birds not observed from the moment they arrived at the mistletoe until the moment they left it. After identification, the birds were classified into four guilds (see Table 1) according to their body size [large frugivores of 60–120 g, small frugivores of 12–20 g; after Mullarney et al. (2000)] and resource acquisition [generalist or opportunist frugivore; after Watson (2012)]. The first group, large generalist frugivores (LG) consisted of large birds which were dietary generalists, feeding on a broad range of fruits during autumn and winter. The second group, small generalist frugivores (SG), was composed of small birds that consumed a diverse range of fruits. The third group, opportunists (O), was formed by small birds, mainly granivores and insectivores that occasionally consumed fleshy fruits. Finally, the last group, small mistletoe visitors (SV), was composed of other small birds, either insectivores or granivores, that visit mistletoe but do not manipulate fruits. The variables Number of visits, Visit duration (minutes) and Fruit-removal rate (fruits per minute) were used to estimate the quantity component of the dispersal effectiveness of each visitor species that could potentially act as a seed disperser.

Once the mistletoe visitors were identified, we analyzed their preferences for different host structures, on both parasitized and non-parasitized trees. First, we divided the trees into three portions: upper third (the treetop), middle third, and the bottom of the tree (lower third). Next, we distinguished between four structures visited by birds: the crown of the pine; and three positions within branches—basal, middle and apical. The branches were divided following the same criteria as for the exploration of seed-deposition and seedling-distribution patterns. The frequency of use of different host structures was used to assign locations with higher probabilities of receiving seeds. This, multiplied by the mistletoe recruitment probabilities previously calculated, was used to characterize the dispersal quality of each bird species. By multiplying the “quantity” and the “quality” components, we calculated the dispersal effectiveness of each bird species. Finally, disperser efficiency was estimated by dividing the quality term by the quantity term.

Seed condition

A sowing experiment was performed to evaluate the suitability of mistletoe seeds after they were internally or

Table 1 List of bird species visiting *Viscum album* subsp. *austriacum* grouped in different guilds depending on their body size and feeding behavior, followed by the sum of mistletoe visits for 306 observation hours, visit duration, mean number of fruits removed per minute, number of ectozoochory and endozoochory events and relative dispersal quantity

Vernacular name (<i>scientific name</i>)	Guild ^a	Σ Visits ^b	Σ Feeding ^c	Min/visit ^d	Fruits/min ^e	Σ Ectozoochory ^f	Σ Endozoochory ^g	Relative quantity ^h
Mistle thrush (<i>Turdus viscivorus</i>)	LG	605	177	2.58 (0.30)	6.27 (1.05)	–	27	1.00
Ring ouzel (<i>Turdus torquatus</i>)	LG	190	42	2.33 (0.18)	8.17 (0.98)	–	3	0.28
Song thrush (<i>Turdus philomelos</i>)	LG	82	20	2.31 (0.17)	8.6 (2.94)	–	1	0.14
Redwing (<i>Turdus iliacus</i>)	LG	15	6	2.45 (0.28)	4.83 (1.88)	–	3	0.025
Common blackbird (<i>Turdus merula</i>)	LG	26	6	1.94 (0.32)	6.10 (1.02)	–	3	0.025
Fieldfare (<i>Turdus pilaris</i>)	LG	12	3	0.99 (0.24)	8.37 (1.80)	–	1	0.009
Blackcap (<i>Sylvia atricapilla</i>)	SG	38	9	1.62 (0.24)	6.31 (1.15)	–	12	0.032
Robin (<i>Erithacus rubecula</i>)	SG	4	3	0.58 (0.08)	4.25 (0.25)	–	–	0.003
Coal tit (<i>Parus ater</i>)	O	37	4	0.50 (0.12)	3 (0.30)	4	–	0.002
Great tit (<i>Parus major</i>)	O	34	3	0.52 (0.13)	3 (0.30)	3	–	0.002
Blue tit (<i>Parus caeruleus</i>)	O	17	9	0.87 (0.20)	1 (0)	9	–	0.003
Long-tailed tit (<i>Aegithalos caudatus</i>)	SV	5	–	–	–	–	–	–
Hawfinch (<i>C. coccothraustes</i>)	SV	1	–	–	–	–	–	–
European goldfinch (<i>Carduelis carduelis</i>)	SV	1	–	–	–	–	–	–
Crested tit (<i>Lophophanes cristatus</i>)	SV	1	–	–	–	–	–	–

^a LG Large generalist frugivore birds (60–120 g), SG small generalist frugivore birds (12–20 g), O opportunist birds (12–20 g), SV other mistletoe visitors

^b Number of mistletoe visits over 306 observation hours, either by direct watching (208 h) or videotaping (98 recording hours)

^c Number of visits in which birds ingested or pecked on fruits

^d Visit duration, in minutes (mean ± SE), was estimated from complete observations of birds visiting mistletoes. When birds stayed more than 3 min, this was the maximum time recorded

^e Number of consumed or pecked fruits per minute (mean ± SE). Complete and partial observations were included to estimate rates of consumption

^f Number of times birds were observed carrying seeds externally and depositing them on pine branches

^g Number of times birds were observed feeding on mistletoe fruits and defecating or regurgitating seeds

^h Quantity values have been estimated as Σ Feeding × fruits/min × min/visit; these were divided by the highest value to calculate relative estimates

externally dispersed. For this, we collected bird-dispersed and non-dispersed mistletoe seeds that were placed on safe sites of *P. nigra* branches. For bird-dispersed seeds, we gathered seeds in the field with clear signs of previous consumption by frugivore birds, expelled either by regurgitation or defecation (simulating an endozoochorous dispersal event); they were mixed to randomization and immediately placed onto tree branches. For non-dispersed seeds, fruits were collected directly from mother plants, the exocarps manually removed (simulating an ectozoochorous dispersal event), and the seeds randomized and placed on host branches. During early March 2010, a total of 900 dispersed seeds and another 900 non-dispersed seeds were inoculated onto twigs (0.99–0.2 cm in diameter) of three limbs of 70 *P. nigra* trees (all alike in age, size, and architecture); then, seeds were monitored every 30–40 days for up to 15 months after planting. In the first monitoring the number of seeds that were lost before their initial

attachment to the host bark was quantified. During seed monitoring, we noted whether a seed was absent or present, and if present, seed condition (alive or dead). We distinguished two stages within live seeds: germinated and established. Germinated included seeds that had started haustoria development, whereas established included seeds with a functional haustoria and emerged cotyledons. Proportions of seed germination (number of germinated seeds vs. the total remaining on tree branches after the first month) and seedling establishment (number of germinated seeds vs. the total remaining on tree branches) were calculated for further analyses.

Statistical analysis

To analyze the distribution of mistletoe seeds and seedlings in the different positions of pine branches, we used generalized linear models (GLMs), assuming a Poisson error

distribution and log function, followed by a Tukey honest significant difference (HSD) pair-wise comparison method. The portion of host used (upper third, middle third or bottom third) and host structures used (crown and branch positions: basal, middle or apical) were compared between avian dispersing guilds (LG, SG and O) with a Pearson's χ^2 -test. Simulated p -values based on 2,000 replicates were used when needed. Germination and establishment success of internally and externally dispersed seeds were compared using GLMs, assuming a binomial distribution of errors and logit-link functions, followed by a Tukey HSD pair-wise comparison method. R software (version 2.10.0, R Development Core Team 2010) was used to perform the statistical analyses, while figures were made in StatView 5.0.1. Tukey's pairwise comparisons with 95 % confidence level were conducted with the multcomp package (Hothorn et al. 2008).

Results

Mistletoe recruitment and seed-deposition sites

Recruitment probabilities differed between branch positions (GLM, $\chi^2 = 66.30$, $df = 3$, $P < 0.0001$), being nil at the thickest sites (basal and middle positions of branches), 0.67 % for apical uncovered twigs and 17.3 % for needle-covered twigs (Fig 1). Seed deposition also differed between branch positions (GLM, $\chi^2 = 649.11$, $df = 3$, $P < 0.0001$), although this followed a different pattern (Fig. 1): 393 seeds (66.16 % of total seeds) were on basal positions, 150 seeds (25.25 %) were on needle-covered apical twigs, only 50 seeds (12.67 %) reached the middle positions, while apical uncovered twigs hardly received one seed (0.002 %).

Identification of mistletoe seed dispersers and seed-deposition patterns

During bird observations, we recorded 15 different bird species that visited fruiting *V. album* subsp. *austriacum*. Eight species were classified as generalist frugivore seed dispersers, three as opportunists, and four as mistletoe visitors that never manipulated fruits. Of the seed dispersers, six were large and two small birds; opportunists and other visitors were all small (Table 1). All seed dispersers swallowed the fruits and defecated or regurgitated the seeds, while opportunists only pecked at the fruit pericarp and externally dispersed mistletoe seeds that adhered to their feathers or beaks. During 98 videotaping hours, for 48 parasitized *P. nigra* trees, we recorded 148 birds visiting mistletoe. For 208 h over 50 days of direct bird observations, we recorded another 361 visits to mistletoe on

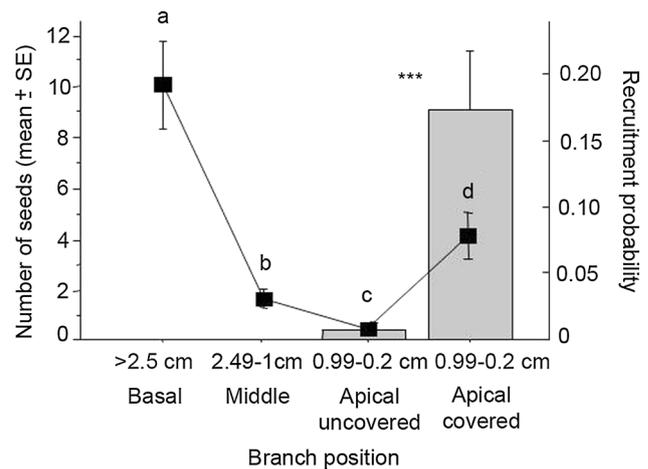


Fig. 1 Pattern of mistletoe seed deposition (black squares) and recruitment probability (gray bars) according to branch diameter. Branches were divided into four categories: >2.5 cm (closest to the tree trunk) (Basal); 2.49–1 cm (Middle); and two apical positions—uncovered, 0.99–0.2 cm (Apical uncovered); and needle-covered, 0.99–0.2 cm (Apical covered). Number of seeds, mean \pm SE, $n = 41$ branches [generalized linear model (GLM), $\chi^2 = 649.11$, $df = 3$, $P < 0.0001$]; differences among branch positions are indicated by different letters. Recruitment probabilities, mean \pm SE, $n = 30$ branches (GLM, $\chi^2 = 66.30$, $df = 3$, $P < 0.0001$); *** $P < 0.0001$

parasitized pines. An additional 564 bird observations were made on non-parasitized trees, which were used to compare the birds' behavioral patterns when mistletoe was absent. Large generalist frugivores accounted for 90 % of the visits, small generalist frugivores for 4.26 % and opportunists for 5.74 %. From all the bird visits, we clearly discerned 282 feeding events (in which birds were observed pecking or swallowing fruits) of which 50 were clear episodes of endozoochory (in which birds defecated seeds after feeding on whole mistletoe fruits), all involving *Turdus* spp. and *Sylvia atricapilla*. Also, we recorded scattered events of ectozoochory by *Parus caeruleus* ($n = 9$ events), *Parus major* ($n = 3$ events) and *Parus ater* ($n = 4$ events). In these cases, as birds pecked at fruits to feed on the pulp, sticky seeds occasionally adhered to their beaks, which were immediately rubbed against pine needles at the tip of the branches to remove the seeds. Quantitatively, thrushes, mainly *Turdus viscivorus*, were major contributors to mistletoe seed dispersal (Table 1).

The three guilds of birds ("other visitors" were excluded as they showed no interaction with mistletoe fruits) differed in the use of host structures ($\chi^2 = 731.76$, $df = 6$, $P < 0.0001$) and frequented different portions of the tree ($\chi^2 = 207.25$, $df = 4$, $P < 0.0001$) (Table 2). Small birds (generalist frugivores and opportunists) differed in the use of the host's structures ($\chi^2 = 50.11$, $df = 3$, $P = 0.0005$) but not in the portion of the tree ($\chi^2 = 2.92$, $df = 2$, $P = 0.272$). Large generalist frugivores mostly frequented

Table 2 Seed-dispersal quality and frequencies of post-foraging host-use of mistletoe visitors, differentiating between three tree portions and four tree structures

Vernacular name (<i>scientific name</i>)	Σ Observations ^a	Tree portion ^b			Tree structure ^c				Relative quality ^d
		Upper third	Middle third	Lower third	Crown	Basal	Middle	Apical	
Mistle thrush (<i>T. viscivorus</i>)	605	0.73	0.26	0.01	0.49	0.13	0.37	0.01	0.54
Ring ouzel (<i>T. torquatus</i>)	190	0.73	0.24	0.04	0.45	0.35	0.19	0.01	0.11
Song thrush (<i>T. philomelos</i>)	82	0.79	0.18	0.02	0.51	0.22	0.27	0.00	0.03
Common blackbird (<i>T. merula</i>)	26	0.50	0.38	0.12	0.23	0.31	0.46	0.00	0.02
Redwing (<i>T. iliacus</i>)	15	0.67	0.33	0.00	0.47	0.53	0.00	0.00	0.00
Fieldfare (<i>T. pilaris</i>)	12	0.67	0.33	0.00	0.33	0.67	0.00	0.00	0.00
Blackcap (<i>S. atricapilla</i>)	38	0.13	0.79	0.08	0.00	0.26	0.45	0.29	0.39
Robin (<i>E. rubecula</i>)	4	0.25	0.50	0.25	0.25	0.00	0.75	0.00	0.00
Coal tit (<i>P. ater</i>)	37	0.03	0.95	0.03	0.00	0.00	0.19	0.81	1.00
Great tit (<i>P. major</i>)	34	0.09	0.79	0.12	0.00	0.00	0.15	0.85	0.96
Blue tit (<i>P. caeruleus</i>)	17	0.06	0.82	0.12	0.00	0.00	0.06	0.94	0.53

^a Number of mistletoe visits over 306 observation hours, either by direct watching (208 h) or videotaping (98 recording hours)

^b Frequency of bird observations classified by the portion of the tree visited: the treetop (*Upper third*), the middle third of the tree (*Middle third*) and the bottom third of the tree (*Lower third*). Frequencies were calculated for each species as the number of visits at each tree portion by the total number of observations

^c Frequency of bird observations classified by the structure of the tree used: the tree crown; and three different positions within branches—basal (closer to the tree trunk); middle; and apical (the branch periphery). Frequencies were calculated for each species as the number of visits at each tree structure divided by the total number of observations

^d The quality term was calculated as the number of bird observations on different branch positions—basal, middle or apical—multiplied by the recruitment probability of each position: basal, 0; middle, 0; apical, 0.173. Recruitment probabilities were experimentally calculated by estimating the proportion of mistletoe seedlings establishing on four different branch positions, where seeds are commonly deposited by birds: basal branch position (diameter < 2.5 cm), middle position (diameter 2.49–1 cm), uncovered apical position (diameter 0.99–0.2 cm), and needle-covered apical position (diameter 0.99–0.2 cm). As most birds visited needle-covered apical positions, we used the recruitment probability of this site for calculations. Quantity values were divided by the highest value to calculate relative estimates

structures located at the treetop (72.5 %), while the majority of small passerines visited medium heights of the pine (82.6 %). About half of the large birds (all *Turdus* species, except for *Turdus merula*) visited mistletoes located at the treetop, and fed on fruits while perched on the crown. After feeding, they kept on watch from the crown, moved to a nearby crown, or moved to basal (20 % of the visits) and middle positions (30 % of the visits) of pine branches. *T. merula*, however, frequently fed on fruits fallen on the ground. Small birds, either generalist frugivores or opportunists, visited the middle portions of the tree. However, while small generalists commonly perched on the middle parts of branches (47 %) and at times used apical twigs (26 %), opportunists followed the opposite pattern, mostly perching on branch tips (85 %) and rarely using thicker structures (15 %). Small opportunists offered the highest quality seed-dispersal services, followed by the large generalist frugivore *T. viscivorus* and the small generalist frugivore *S. atricapilla* (Table 2). Overall, *T. viscivorus* was the most effective seed disperser, while opportunist species were the most efficient dispersers (Table 3).

Birds behaved similarly in parasitized and non-parasitized pines regarding the portion of the host used (high,

middle, low) by each group of dispersers (LG, $\chi^2 = 0.37$, $df = 2$, $P = 0.83$; SG, $\chi^2 = 10.17$, $df = 2$, $P = 0.062$; O, $\chi^2 = 2.28$, $df = 1$, $P = 0.13$; the low position was eliminated from the latter analysis because of the lack of data). No differences were found, either, in the structures (crown or branch positions: basal, middle and apical) preferred by birds when the host was parasitized or not (LG, $\chi^2 = 16.73$, $df = 3$, $P = 0.051$; SG, $\chi^2 = 2.54$, $df = 3$, $P = 0.11$; O, $\chi^2 = 1.28$, $df = 3$, $P = 0.26$) (Fig. 2). Overall, the preferred structure by LG was the tree crown, i.e., 41 % of the visits in parasitized trees and 53 % in non-parasitized.

Seed condition

With regard to seed handling, no differences were found between treatments in seed germination and seedling establishment. Of the seeds remaining on host branches (disregarding seeds that failed in their initial attachment to host branches), 69 % of internally dispersed seeds and 71.4 % of those externally dispersed germinated (GLM, $\chi^2 = 0.17$, $df = 1$, $P = 0.68$), while 8.2 % of externally dispersed seeds and 5.6 % of internally dispersed seeds successfully established (GLM, $\chi^2 = 2.24$, $df = 1$, $P = 0.14$).

Table 3 Seed-dispersal effectiveness (quality × quantity) and efficiency (quality/quantity) of mistletoe visitors

Vernacular name (<i>scientific name</i>)	Quantity ^a	Quality ^b	Relative effectiveness ^c	Relative efficiency ^d
Mistle thrush (<i>T. viscivorus</i>)	2,863.26	2.87	1.00	0.00
Ring ouzel (<i>T. torquatus</i>)	799.52	0.59	0.06	0.00
Song thrush (<i>T. philomelos</i>)	397.32	0.15	0.01	0.00
Common blackbird (<i>T. merula</i>)	71.00	0.08	0.00	0.00
Redwing (<i>T. iliacus</i>)	71.00	0.00	0.00	0.00
Fieldfare (<i>T. pilaris</i>)	24.86	0.00	0.00	0.00
Blackcap (<i>S. atricapilla</i>)	92.00	2.02	0.02	0.02
Robin (<i>E. rubecula</i>)	7.40	0.02	0.00	0.00
Coal tit (<i>P. ater</i>)	6.00	5.24	0.00	0.81
Great tit (<i>P. major</i>)	4.68	5.05	0.00	1.00
Blue tit (<i>P. caeruleus</i>)	7.83	2.77	0.00	0.33

^a The quantity term was estimated as the number of visits in which birds fed on mistletoe fruits, multiplied by the number of fruits consumed per minute and the duration of the visit (minutes)

^b The quality term was calculated as the number of bird observations on different branch positions—basal, middle or apical—multiplied by the recruitment probability of each position: basal, 0; middle, 0; apical, 0.173. Recruitment probabilities were experimentally calculated by estimating the proportion of mistletoe seedlings establishing on four different branch positions, where seeds are commonly deposited by birds: basal branch position (diameter < 2.5 cm), middle position (diameter 2.49–1 cm), uncovered apical position (diameter 0.99–0.2 cm), and needle-covered apical position (diameter 0.99–0.2 cm). As most birds visited needle-covered apical positions, we used the recruitment probability of this site for calculations

^c Seed-dispersal effectiveness is defined as the probability of a seed being handled by a particular bird that reaches a “safe site.” It was calculated as the product of the quantity and the quality terms, then divided by the highest value to calculate relative estimates

^d Disperser’s efficiency is a measure of the number of dispersed seeds relative to the number of handled seeds. It was calculated as the ratio of quality and quantity terms, and then divided by the highest value to calculate relative estimates

Discussion

This study explicitly examines the poorly explored topic of how generalist birds, in the absence of specialized dispersers, provide seed dispersal for a parasitic plant with strong recruitment constraints. We first identified the potential group of seed dispersers of *V. album* subsp. *austriacum* in a Mediterranean mountain of southeastern Spain. Then, by linking species-specific behavior to seed-dispersal patterns and to demographic consequences for the parasite in different microhabitats, we discovered that this mistletoe can rely on unspecialized vectors to disperse seeds to narrowly defined safe sites.

A broad and diverse group of 11 bird species (except for strict granivores or insectivores) disperse seeds of *V. album* subsp. *austriacum* in Sierra de Baza. Any bird can be a potential disperser, as the seeds do not need to be ingested to germinate. This flexibility guarantees that *V. album* subsp. *austriacum*, irrespective of the composition and abundance of the avian community in a pine forest, will satisfy its seed-dispersal needs. Regardless of the dispersal mode, all vectors have to deposit seeds on proper safe sites—narrowly limited to thin branches of the tree periphery—in order to exert a real impact on mistletoe demography. Small epizoochorous and endozoochorous birds disperse, respectively, by rubbing their beaks against pine

needles or directly defecating on twigs. Meanwhile, large endozoochorous birds disperse by haphazardly and abundantly defecating or regurgitating from the treetop. In short, any bird able to deposit a seed on a suitable site qualifies as a seed disperser.

Among endozoochorous vectors, large generalist frugivores constitute the most effective dispersal guild; however, they provide low-efficiency services, consuming numerous fruits in order to suitably disperse a single seed. Their preferences for firm and robust structures as perching sites finally result in abundant seed rain with most seeds reaching basal, thicker parts of branches. At these sites the seeds have no chance of survival, the haustorium being unable to penetrate the xylem of the host, and furthermore are likely to become desiccated by extreme summer temperatures or to be depredated (Melado and Zamora 2014). On the other hand, as thrushes provide high-quantity dispersal services, the few times (1.1 %) they used apical sites of branches led to a considerable number of favorable dispersal events, which notably contributed to the final recruitment of the parasite. In the light of these results, large generalist frugivores might act as effective seed dispersers whenever mistletoe fruit availability is sufficient to offset their high inefficiency. *V. album* subsp. *austriacum* can afford this wasteful dispersal mechanism by producing abundant and constant crops of small fruits over most of the fruiting season (Zuber 2004). In contrast, although small generalist frugivores

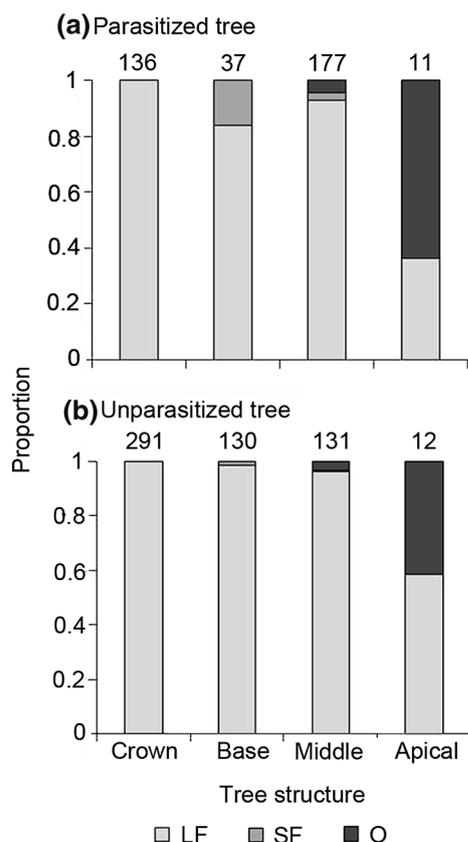


Fig. 2 Post-foraging microhabitat use of mistletoe seed dispersers on parasitized and non-parasitized trees. Shown is the relative use frequency of different host structures by major groups of mistletoe seed dispersers. Tree structures are indicated as: treetop (Crown); and three different positions within branches—closest position to the tree trunk (*Basal*), middle position (*Middle*), branch periphery (*Apical*). Groups of seed dispersers are shown as large generalist frugivores (LG), including *Turdus viscivorus*, *Turdus torquatus*, *Turdus philomelos*, *Turdus merula*, *Turdus iliacus* and *Turdus pilaris*; small generalist frugivores (SG), including *Sylvia atricapilla* and *Erithacus rubecula*; opportunists (O), including *Parus ater*, *Parus major* and *Parus caeruleus*. Pearson's χ^2 -test, LG, $\chi^2 = 16.73$, $df = 3$, $P = 0.051$; SG, $\chi^2 = 2.54$, $df = 3$, $P = 0.11$; O, $\chi^2 = 1.28$, $df = 3$, $P = 0.26$. Sample sizes are shown above the bars

provide better quality and more efficient dispersal, they were so scarce that their contribution to the parasite was almost negligible during our study. On the other hand, opportunist tits can be considered very efficient dispersers by moving seeds externally and directly to safe sites of the tree periphery. This external seed transport by dietary generalists and opportunist frugivores has been frequently reported among *Viscaceae* mistletoes (e.g., Punter and Gilbert 1989; Restrepo et al. 2002), which may confer several advantages to the parasite. First, single deposition onto safe sites may help seeds to escape high post-dispersal seed predation—both because of lower seed densities (Davidar 1983) and, particularly in this system, because of the higher protection of seeds under needle coverage, where they have optimum recruitment conditions

(Mellado and Zamora 2014). Secondly, once seedlings become established, sibling competition may be diminished (Davidar 1983). In this sense, the demographic consequences of tits would be akin to those of mistletoe specialists, if not for the scarcity of the dispersal events. This indicates that morphological adaptations do not necessarily confer advantages for mistletoe dispersal over less specialized vectors, and further highlights the great importance that unexpected dispersal agents can have for the demography of a plant (Calviño-Cancela 2002; Heleno et al. 2011; Frick et al. 2013).

An interesting finding is that birds visit parasitized as well as non-parasitized pines, behaving similarly on both. This, coupled with the large home ranges of dispersal vectors (especially thrushes) and the wide and heterogeneous range of seed dispersers, may increase the overall dispersal effectiveness of the mistletoe population, with several bird species moving seeds at different distances; this ensures local population functions, including reinfection processes, and the establishment of new populations or expansion of the plant's range, which might relieve host populations from intense local reinfection processes (Watson and Rawsthorne 2013). *V. album* subsp. *austriacum*, being able to cope with massive seed losses (by producing large fruit crops) and to accept any dispersal mechanism (either endozoochorous or ectozoochorous), can achieve effective dispersal by an unspecialized dispersal group. In conclusion, the mutualistic interaction between the European mistletoe and zoochorous birds is a generalized system; almost all birds feeding on fruits can act as effective seed dispersers. Plant dispersal success relies on the correspondence between a large fruit crop and a diverse guild of endozoochorous and/or ectozoochorous dispersal vectors. These generalistic features enable the spatio-temporal replacement of dispersers, allowing the presence of *Viscaceae* species in geographical ranges lacking specialist dispersers, such as occur in Europe.

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