

A Host Creates an Enemy-free Space for Mistletoes by Reducing Seed Predation Caused by a Woodboring Beetle: A Hypothesis

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ABSTRACT

Parasitic plants rely on host plants for nutrition. The number of host species varies largely between groups, from single species or genus to hundreds of species. Relative abundance of the host and evolutionary history are the main requisites for parasitic plants to develop specificity to abundant hosts. In the present study, we suggest a novel mechanism by which a hemiparasitic mistletoe can develop local specificity mediated by its host. First, we describe a novel interaction in which a woodboring beetle (*Hypothenemus obscurus*) preys on mistletoe seeds (*Psittacanthus plagiophyllus*) attached to tree branches. This beetle preys more frequently on seeds deposited on branches of non-host species than on branches of its unique local host species (53 percent on average vs. 26 percent respectively). We hypothesize that local host specificity for this mistletoe could be partly mediated by beetle-host incompatibility, since the host offers a predation-free space in which mistletoes have better chances to grow. Furthermore, that the exceptional gum exudates produced by this unique host species minimize beetle attacks on branches, thus reducing predation of mistletoe seeds. This novel tritrophic interaction opens an avenue for research on macroscopic host-specificity mechanisms that occur at the level of the host bark and that have been poorly studied by plant ecologists.

Abstract in Portuguese is available in the online version of this article.

Key words: enemy-free space; host compatibility; host gum exudate; plant parasites; post-dispersal seed predation.

HOST SPECIFICITY IN PARASITIC PLANTS ARISES FROM TWO INTERACTING PROCESSES: the capacity of the parasite to recognize and infect different hosts, and the ability of the hosts to defend, both mechanically and chemically, their tissues from parasites (Shen *et al.* 2006). Several studies have shown that chemical compatibility between the host and parasite is the key for promoting host-specificity in root parasites (Press *et al.* 1990, Thorogood & Hiscock 2010). For aerial parasites, such as mistletoes, a variety of other mechanisms have been invoked to explain local host-specificity. Most of these are passive; for example if birds prefer to perch on taller hosts then host height could help explain specificity (Aukema & Martinez Del Rio 2002). Others include nitrogen content, as mistletoes prefer nitrogen-richer hosts (Ehleringer *et al.* 1985), relative host abundance, as abundant trees have a higher chance of interacting with mistletoes (Norton & Carpenter 1998, Norton & De Lange 1999), and mistletoe-host compatibility, because chemical recognition between mistletoe and host is required to promote specialization (López de Buen & Ornelas 2002). However, mechanisms that occur at the level of the host bark are poorly studied (Rodl & Ward 2002). An enormous diversity of arthropods live on plant branches (within and above the bark) and can harm mistletoe seeds and seedlings, but nothing is known about their potential influence on mistletoe-host

prevalence or host specificity. We described a new interaction in which a woodboring beetle preferentially preys on mistletoe seeds attached to non-host branches. Furthermore, we raised the hypothesis that the strong local mistletoe-host specificity found in our study system could be influenced, at least in part, by differences in seed predation by the beetle.

The study was carried out in a transition between a woody tropical savanna and a sandy beach on the right margin of the Tapajos River, Santarém, Brazil (2°31'S, 59°00'W). *Psittacanthus plagiophyllus* is a shrubby hemiparasitic mistletoe that occurs in northern South America and central Brazil (Kuijt 2009). This species is strongly specialized to the cashew tree, *Anacardium occidentale*, at the local scale (Fadini & Lima 2012) (*i.e.*, seeds only germinate in this species), which is rare for mistletoes (Fadini 2011). Fruits are dispersed primarily by the Plain-crested Elaenia (*Elaenia cristata*) (Fig. 1A), which regurgitates intact seeds on tree branches (Fig. 1B).

EXPERIMENT 1: INCIDENCE AND ABUNDANCE OF BEETLES IN MISTLETOE SEEDS ATTACHED TO DIFFERENT TREE SPECIES.—We planted mistletoe seeds manually on slender branches (10–15 mm) of six tree species. These species — *Anacardium occidentale*, *Bowdichia virgilioides*, *Byrsonima crassifolia*, *Himatantbus fallax*, *Lafoensia paccari*, and *Salvertia convallariaeodora* — were selected based on their high relative abundance in the study site. Five seeds were planted per branch, with two branches per individual and ten

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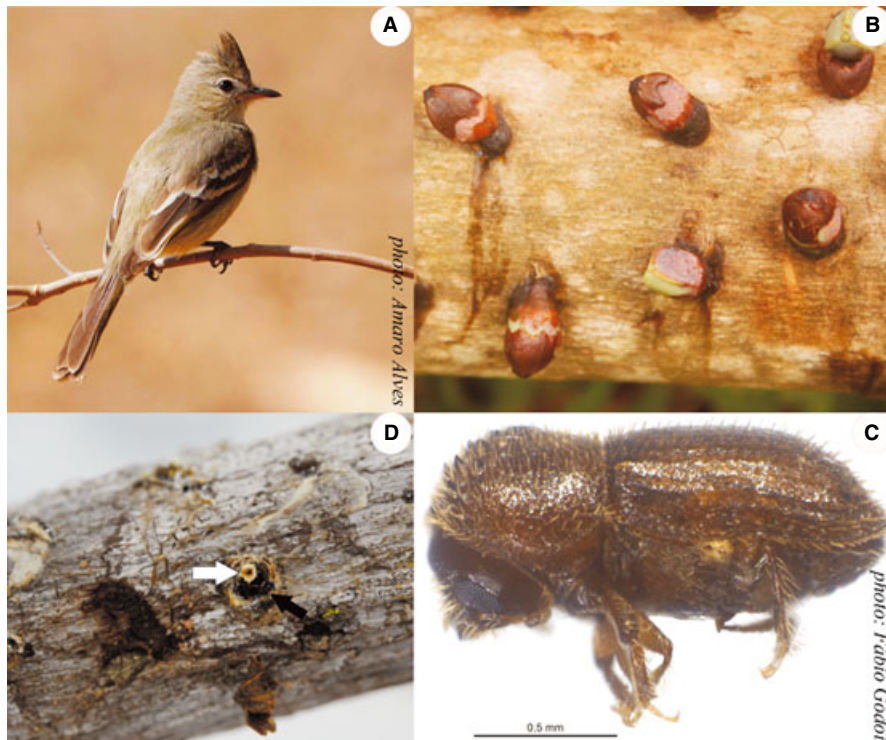


FIGURE 1. *Elaenia cristata*, one of the main seed dispersers of *Psittacanthus plagiophyllus* (A), regurgitate seeds of this mistletoe on a tree branch (B). *Hypothbenenus obscurus* (C) become lodged on a host branch and access the seed through a tube-like structure (white arrow) (D). Region in which a seed of *P. plagiophyllus* was attached to a host branch (black arrow). Color version available in online Supporting Information.

individuals per species (*i.e.*, 100 seeds per tree species). A previous experiment showed that 75 percent of hand-planted seeds were alive in the first month after inoculation (R. Fadini, unpubl. data). Therefore, we expect that not passing seeds through bird guts would not reduce survivorship and germination. Two months after inoculation, we counted the number of dead seeds per branch and selected one dead seed per branch (*i.e.*, the most distal) to evaluate the cause of mortality (20 seeds per host species). Beetles of an unidentified species were found inside the seeds (Fig. 1C). We opened the seeds manually and counted the number of individuals of this species using a stereomicroscope with 20 \times magnification. We estimated the proportion of predation by beetles by multiplying the proportion of preyed seeds by the proportion of dead seeds.

EXPERIMENT 2: PRE- OR POST-DISPERSAL SEED PREDATION?.—Differences in seed predation among seeds planted in different host species in the first experiment (see *Results*) indicated that seed predation occurred after dispersal. We conducted another experiment to confirm this pattern and identify the underlying mechanism, using seeds directly collected from the mother plant and other alive, recently dispersed seeds, naturally deposited on live host branches (*A. occidentale*) by seed dispersers (dispersed seeds). We manually removed seeds from the fruit epicarps and fixed them on dead branches of host trees (planted seeds). We also collected mistletoe fruits (intact fruits) from eight host trees to

control for possible effects of epicarp removal on non-dispersed seeds during inoculation. All types of mistletoe seeds were kept in a common garden exposed to natural conditions. We obtained non-dispersed seeds from single mistletoes that were randomly picked within 26 host trees. Twenty-percent of all ripe fruits were collected from each mistletoe. We collected dispersed seeds from branches of 25 host trees, a maximum of 50 seeds per tree. Seeds did not germinate in dead branches; thus, once they had dried out, we hand-opened the diaspores and checked for beetles. We considered seeds to be preyed upon only if they contained beetles or galleries similar to those found in diaspores with beetles (indirect evidence). Other types of hole found in the seeds were excluded from the analysis. Our observations indicated that predation by other insects (*i.e.*, Diptera) was negligible in our study site. This may be not the case elsewhere (Uchôa-Fernandes *et al.* 2012).

We used a generalized linear model with a negative binomial error distribution to predict the proportion of seeds preyed upon per host. Seed category (dispersed, planted, and intact) was the predictive variable and the number of seeds collected was the covariate. We made one statistical analysis using indirect signals of seed predation as the response variable (see above), and present the minimum adequate models without overdispersion (Crawley 2007). Results are presented as mean \pm SD.

In the first experiment (with mistletoe seeds planted in different tree species), the mortality of mistletoe seeds was close

to 100 percent on all but one species, *A. occidentale* (Table 1). Seed predation was caused by *Hypotbenemus obscurus* (Scolytinae), and was highest in *Byrsonima crassifolia* and lowest in *A. occidentale*. In general, seed predation on *A. occidentale* was half than the average of all other tree species tested (one sample *t* test: 26 vs. 53 percent on average; $t = 5.72$, $df = 4$, $P = 0.004$) (Table 1).

In the second experiment (conducted only in cashew trees), we confirmed that differences in seed predation among tree species in the first experiment occurred after dispersal, probably influenced by differences in beetle abundance. Our many observations of live seeds indicated that beetles start preying on them at their point of attachment to the host, constructing tunnel-like structures from their own chambers to access the cotyledons (Fig. 1D). As beetle eggs, larvae, and adults inside seeds were found, we assumed that beetles might complete at least one reproductive cycle within the seed, acting both as seed parasites and seed predators. We checked for beetle presence in 2463 diaspores: 933 dispersed seeds, 273 intact fruits, and 1257 planted seeds. We found direct evidence of beetle presence in 9.13 ± 2.2 percent of the dispersed seeds (63 seeds), 0 percent of the intact fruits and 0.15 percent of the planted seeds (four seeds). When considering direct and indirect evidence of beetles (both beetles and galleries), the average proportions of seeds preyed upon was 18.9 ± 3.8 percent (144 seeds), 0.7 ± 0.5 percent (two seeds), and 1.0 ± 0.2 percent (10 seeds) respectively. The minimum adequate model included just the seed category as a factor. Seed predation increased significantly for dispersed seeds ($\eta^2 = 2.9 \pm 0.3$, $\chi = 9.2$, $P < 0.001$) and decreased significantly for both intact fruits ($\eta^2 = -3.2 \pm 0.7$, $\chi = -4.4$, $P < 0.001$) and planted seeds ($\eta^2 = -3.6 \pm 0.52$, $\chi = -6.9$, $P < 0.001$).

Direct evidence of woodboring beetles (*H. obscurus*) in seeds of *Psittacanthus plagiophyllus* attached to tree branches represents the first record of post-dispersal predation of mistletoe seeds by a woodboring insect and one of the few records of post-dispersal predation of mistletoes seeds in general (Sargent 1995, Yan & Reid 1995). An alternative hypothesis that could arise is that beetles only predate seeds already dead. However, this is not the case because all seeds collected from the field were recently dispersed. Therefore, beetles are the primary seed predators, not secondary messengers of death. Seeds of *Psittacanthus* figure among the largest in mistletoes (Kuijt 2009) and may offer enough resources for beetles to complete their development. For

instance, a single seed may accommodate up to five adult seed predator beetles.

The other two *Psittacanthus* species with seeds of comparable size, which occur in savanna patches within our study site, did not have their seeds preyed upon by the beetle. Fadini (2011) showed that *P. plagiophyllus* did not germinate until 1 mo or more after dispersal, whereas the other two species (*P. eucalyptifolius* and *P. biternatus*) germinate within a few hours or days. Limited resource availability due to fast germination and early expansion of cotyledons in these last two species may explain the absence of beetles.

By showing that seeds of *P. plagiophyllus* attached to branches of non-host species were two times more preyed upon than seeds attached to branches of their single host, we suggest that this large difference in seed predation on different hosts may represent a mechanism for local host specificity in this mistletoe species, maybe hiding a hitherto overlooked process. Furthermore, we suggest that this difference in seed predation is related to the response of the main host plant to bark penetration — the host's defense mechanism against woodborers (not shown) paradoxically allows infection and establishment by mistletoes.

Cashew gum exudate can be a strong insecticide, reducing oviposition in some insect species (Marques *et al.* 1992). Cashew trees may also deter woodboring beetles from penetrating into their branches mechanically by sticking beetles with their gum. Knowledge of a similar mechanism involving bark beetles and conifers is widespread (Paine *et al.* 1997, Franceschi *et al.* 2005). Although it is not completely efficient (resin defenses crash during mass attacks by beetles), it assures conifers a relative success and a long history of stability and coevolution with some species of bark beetles (Franceschi *et al.* 2005). Many species of mistletoes can also incorporate the host's secondary compounds in their own defense strategies against herbivores and pathogens (Atsatt 1977). To draw a parallel with our study system, hosts can help mistletoes against herbivores or pathogens by creating an enemy-free space for mistletoes, which can lead to mistletoe-host specificity.

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TABLE 1. Mortality of seeds of *Psittacanthus plagiophyllus* planted manually on branches of different tree species in Alter do Chão, Para, Brazil, and checked 2 mo after the beginning of Experiment 1.

Tree species	Proportion of seeds dead <i>in situ</i> (A)	Proportion of dead seeds preyed on by beetles (B)	Proportion of seeds preyed on by beetles (A x B)	# of beetles per preyed seed
<i>Anacardium occidentale</i>	0.75	0.35	0.26	1.71 ± 1.49
<i>Bowdichia virgilioides</i>	0.93	0.45	0.42	2.44 ± 1.13
<i>Byrsonima crassifolia</i>	0.96	0.7	0.67	1.57 ± 0.94
<i>Hymatanthus fallax</i>	0.99	0.55	0.54	2.09 ± 1.22
<i>Lafoensia pacari</i>	0.99	0.61	0.6	1.36 ± 0.5
<i>Salvertia convallariaeodora</i>	0.94	0.44	0.42	1.5 ± 0.92

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