

Does attraction to frugivores or defense against pathogens shape fruit pulp composition?

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Abstract Fruit traits evolve in response to an evolutionary triad between plants, seed dispersers, and antagonists that consume fruits but do not disperse seeds. The defense trade-off hypothesis predicts that the composition of nutrients and of secondary compounds in fruit pulp is shaped by a trade-off between defense against antagonists and attraction to seed dispersers. The removal rate model of this hypothesis predicts a negative relationship between nutrients and secondary compounds, whereas the toxin-titration model predicts a positive relationship. To test these alternative models, we evaluated whether the contents of nutrients and secondary compounds can be used to predict fruit removal by mutualists and pathogens in 14 bird-dispersed plants on a subtropical island in São Paulo state, southeastern Brazil. We selected eight to ten individuals of each species and prevented fruit removal by covering four branches with a net and left fruits on four other branches available to both, vertebrate fruit consumers and pathogens. The persistence of ripe fruits was drastically different among species for bagged and open fruits, and all fruit species persisted

longer when protected against seed dispersers. We found that those fruits that are quickly removed by vertebrates are nutrient-rich, but although the attack rate of pathogens is also high, these fruits have low contents of quantitative defenses such as tannins and phenols. Thus, we suggest that the fruit removal rate by seed dispersers is the primary factor selecting the levels of fruit defense. Likewise, nutrient-poor fruits have low removal of seed dispersers and low probability of attack by pathogens. These species retain ripe fruits in an intact condition for a prolonged period because they are highly defended by secondary compounds, which reduce overall attractiveness. However, this strategy might be advantageous for plants that depend on rare or unreliable dispersers.

Keywords Fruit pathogens · Fruit removal · Secondary compounds · Plant–animal interactions

Introduction

To overcome the constraints imposed by immobility, many plants produce fruits and flowers to recruit animal vectors for reproduction and dispersal. An often overlooked disadvantage of this strategy is that the nutritional rewards of fruits and flowers also attract antagonists that consume those rewards without providing reproductive benefits to the plant. The evolution of fruit and flower traits is thus assumed to respond to the sum of selective pressures exerted by mutualists and antagonists (Cipollini and Levey 1997a; Whitney and Stanton 2004). Teasing apart the relative influence of each consumer type on plant traits is hindered by the complexity and diffuse nature of their interactions with plants (Irwin et al. 2003; Whitney and Stanton 2004).

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The interactions between fruiting plants, mutualists and antagonists are usually mediated by secondary metabolites. These compounds have complex functions in ripe fruits because fruit consumption can be either beneficial or detrimental to plants, depending on whether the consumer disperses or destroys the seeds (Levey et al. 2006). The defense trade-off hypothesis is the most influential framework to explain the relative contents of nutrients and secondary compounds in fruit pulp (Cipollini and Levey 1997a, b). This hypothesis is based on the assumption that secondary compounds in ripe fruits represent a trade-off with respect to defense against damaging agents and palatability for dispersers (Herrera 1982; Cipollini and Levey 1997b). There are two alternative models of this hypothesis leading to contrasting predictions on the relative amount of nutrients and secondary compounds in fruits. The removal rate model suggests an inverse relationship between nutrients and secondary compounds because nutrient-rich fruits should be quickly removed and thus should require few chemical defenses. In contrast, the nutrient/toxin titration model posits a positive correlation because nutrient-rich fruits should be profitable enough to allow for the retention of higher levels of defense (Cipollini and Levey 1997b).

To date, only Schaefer et al. (2003) has tested the two models of fruit biochemistry interspecifically, in a study comparing 33 plant species. This study supported the removal rate model concluding that nutrients stimulated fruit removal by seed dispersers while secondary compounds deterred it. However, fruit consumption by antagonists was not measured in this study and other similar studies on limited numbers of fruit species (Cipollini and Stiles 1993; Cipollini and Levey 1997a). Even though, Tang et al. (2005) also excluded seed dispersers from fruits and explained the variation in persistence time of these fruits with variation in defense against pathogens, they did not measure the amount of secondary compounds, and therefore the predictions of the models on fruit biochemistry could not be directly tested.

To test the alternative models on fruit composition we performed a controlled experiment on the relative fruit removal rates of mutualists and pathogens. To measure fruit removal by pathogens we excluded seed dispersers from fruits and compared persistence time of these fruits to control fruits that were freely accessible on the plant. By excluding seed dispersers we also excluded vertebrate seed predators. However, we minimized this effect by selecting species that are very rarely consumed by vertebrate seed predators such as parrots and pigeons. In a broad study of fruit-eating birds in the Atlantic forest, seed predators were only responsible for 2.3% of the total visits of frugivorous birds (Pizo 2004). The mesh size of the nets that we used to cover fruits did not exclude fungi, bacteria, and small insects. These organisms are the most ubiquitous seed pre-

dators (Cipollini and Levey 1997c), and exert indirect negative effects on plant fitness, because fruit rot deters frugivores and direct negative effects through seed destruction (Cipollini and Levey 1997c). However, the importance of these pathogens has often been ignored in theories of fruit–disperser interactions (but see Janzen 1977; Herrera 1982; Cipollini and Stiles 1992a; Cipollini and Levey 1997c). We therefore tested whether fruit consumption explains fruit removal rates of vertebrates and attack rate of invertebrates and pathogens. Based on the defense trade-off hypothesis we predicted that fruits rich in secondary compounds should have higher persistence time (i.e., lower attack rates by insects, fungi, and bacteria) and lower fruit removal by vertebrates than fruits with low contents of secondary compounds. If the removal rate model explains fruit pulp biochemistry, we predicted that nutrient-rich fruits should have lower persistence time because they are quickly removed by vertebrate seed dispersers. Conversely, fruits that are removed slowly should be highly defended and consequently persist for longer periods of time. If the alternative nutrient/toxin titration model explains fruit pulp contents, we expected that all fruits are under selection pressure for high fruit defense, but only nutrient-rich fruits are able to retain high levels of secondary metabolites.

We tested these predictions of the alternative models in a sub-sample of a tropical plant community. We evaluated fruit morphology, nutrient contents, and secondary compounds of these species and compared removal rates of vertebrate fruit consumers and pathogens. The specific goals of this study were: (1) to test whether nutrient rich fruits are more attractive to both frugivores and pathogens; (2) to evaluate if strongly protected fruits persist longer when seed dispersers are excluded, as suggested by the defense trade-off hypothesis and (3) to evaluate which alternative model, the removal rate or toxin titration, better explain the pulp content of ripe fruits.

Materials and methods

Study site

Fieldwork was carried out on Ilha do Cardoso, a land-bridge subtropical island, in São Paulo state, southeastern Brazil (25° 05' S; 47° 53' W). The vegetation of the island is composed exclusively of Atlantic rain forest represented by five different types: mangroves, dune vegetation, restinga forest, lowland tropical rainforest, and highland tropical rainforest (Noffs and Baptista Noffs 1982).

We conducted the experiments in restinga forest (sandy forest) and in lowland tropical rainforest. Restinga is an ecosystem of the Atlantic rainforest biome that belongs to the group of pioneer formations with marine influence and

that is distributed over a quaternary coastal plain (Ab'Saber 1955). The vegetation is characterized by low and medium canopy height (3–15 m) composed of trees with branched trunks (Sugiyama 1998; Couto and Cordeiro 2005). The lowland tropical Atlantic rain forest is characterized by an understory level and a relatively continuous and dense canopy that can reach 20 m with large amounts of epiphytes and vines.

The climate is generally warm and wet throughout the year but may be divided into two seasons: a cold and drier period from April to August when temperature may drop to nearly 13°C and rainfall is ca. 500 mm, and a warm and rainier period from September to March when temperature may reach 32°C and rainfall 1,800 mm (Oliveira-Filho and Fontes 2000).

Study species

This study examined fruit removal and characteristics of 14 plant species, all originating from different families. We used a conservative approach selecting species from different families in order to minimize phylogenetic effects, because most differentiation of nutritional fruit traits occurs at the level of genera (Jordano 1995). We selected common shrubs ranging from 1 to 4 m, and two species of vines (*Smilax brasiliensis* and *Cissampelos pareira*). We selected species for which we had detailed information on the suite of seed dispersers (Francisco and Galetti 2001, 2002; Pizo et al. 2002; Manhães 2003; Scherer et al. 2007, as well as personal observations). All fruits were ornithocoric ranging from 3.8 to 14.4 mm in size. The main seed dispersing vertebrates are small birds, such as tanagers, thrushes, and flycatchers (see Table 1). Parrots and pigeons, which are important seed predators at Ilha do Cardoso, do not forage in the forest understory and we did not find any evidence of small mammals consuming the fruits of the studied species. Owing to detailed information on the range of frugivores that regularly consume these fruits, we considered insects and pathogens, such as fungi and bacteria, the main seed predators of the species that we evaluated.

Fruit removal

We selected 8 to 10 individuals of each species to evaluate fruit removal. The experiments took place in late summer (February and March) of 2005 and 2006, but observations extended for many months for some species. In each individual plant we covered four branches with nets and marked four other branches but left fruits open to seed dispersers (see Tsahar et al. 2002). The number of fruits present on each branch and those that had fallen into the nets in the bagged treatment was counted every 5 days, except for species with a long persistence time, like the palm *Geonoma*

schottiana (Arecaceae), which we checked every fortnight. The number of fallen fruits was used as a surrogate for pathogen attack, because alterations of fruit tissue via fungal degradation can decrease persistence time by hastening abscission (Cipollini and Stiles 1992b). Even though we cannot distinguish between natural abscission and fruit rot, a large proportion of fruits fallen into the nets showed signs of attack by pathogens (small wounds, color changes). Thus, we assumed that fruit rot was the primary reason for abscission. We always set up the experiment before the fruits became ripe and began the experiment at maturation. Individual fruits in the bagged treatment were classified as “fallen”, if they were found in the net, or “persisted”, if they were still attached to the plant. Fruits of the open treatment were classified as “removed”, if they disappeared from the plant, or “not removed”, if they persisted on the plant. In each treatment we calculated the mean persistence time of fruits for each individual and used it for further analysis. To calculate removal efficiency we subtracted the mean number of fruits fallen into the nets from the mean number of missing fruits on the exposed branches and computed removal efficiency as the percentage of the total fruit crop from each tree (for details, see Tsahar et al. 2002).

We counted the number of fruits for all individuals. When crop size was small, all fruits were counted, otherwise crop size was estimated dividing the plant into four equal sections and counting all fruits present in one part and extrapolating it to the other ones. We also measured plant height and the distance from other fruiting trees of the same species (d1) and of fruiting trees of different species (d2) in an area of 15 m radius to delineate a patch of fruit resources, as it might be perceived by birds (Saracco et al. 2004).

Morphological traits

We collected 30 fruits of at least three different individuals to study fruit morphology. For each fruit we recorded: (1) length and diameter of the fruit and seeds; (2) fruit dry and fresh mass; (3) seed mass; (4) number of seeds per fruit. From these measurements we calculated two ratios: (1) relative yield of a fresh fruit (dry mass of the pulp/fresh mass of whole fruit); (2) pulp to seed ratio (dry mass of pulp/mass of the seeds).

Chemical analyses

For the chemical analyses, fruit pulp from at least three individuals of each species was frozen until the moment of analysis. Proteins were determined according to the Kjeldahl method, in which total protein is calculated by multiplying total nitrogen by 6.25 (Jeffery et al. 1989). Lipid contents were determined according to the macro-gravimetric method (Bligh and Dyer 1959) and the contents of

Table 1 Bird species that consume the fruits of the studied species

Frugivores	St	Gs	Mr	Gb	Ea	Op	Or	Cp	Ru	Mm	Go	Pn	Sb	Tb
Characidae														
<i>Penelope superciliaris</i>		2						2						
Mimidae														
<i>Mimus saturninus</i>						4,8								
Psittacidae														
<i>Pyrrhura frontalis</i>	7													
Ramphastidae														
<i>Selenidera maculirostris</i>		7												
Thraupidae														
<i>Nemosia pileata</i>									3*					
<i>Ramphocelus bresilius</i>	2		2	2						9			2	
<i>Schistochlamys ruficapillus</i>		5			4			5	6*					
<i>Stephanophorus diadematus</i>	7													
<i>Tangara cayana</i>							2		3*					
<i>Thraupis sayaca</i>	2		2	2	2	4,8	2		3*,8				2	
<i>Thraupis ornata</i>	7													
Turdidae														
<i>Turdus albicollis</i>						4,8			8		8	9		
<i>Turdus amaurochalinus</i>					8*	4,8		2	8		2			1
<i>Turdus leucomelas</i>						4			3*					
<i>Turdus rufiventris</i>						4,8			8		2			1
Tyrannidae														
<i>Elaenia flavogaster</i>						4,8			8					
<i>Elaenia obscura</i>										9				
<i>Elaenia sp.</i>						4,8			8				2	1
<i>Empidonomus varius</i>						4								
<i>Megarhynchus pitangua</i>									3*					
<i>Myiarchus tyrannulus</i>						4				9				1
<i>Myiodynastes maculatus</i>				2		4							2	
<i>Myiozetetes similis</i>	2					4							2	
<i>Pitangus sulphuratus</i>	2					4,8								
<i>Tyrannus melancholicus</i>						4			3*					
<i>Tyrannus savana</i>	2		2			4							2	1
Picidae														
<i>Colaptes melanochorus</i>									3*					
Pipridae														
<i>Antilophia galeata</i>									3*					
<i>Carpornis cucullatus</i>										6*	6		6*	
Thamnophilidae														
<i>Thamnophilus caeruleus</i>	7													
Vireonidae														
<i>Vireo olivaceus</i>									3*					

St *Schinus terebinthifolius*; Gs *Geonoma schottiana*; Mr *Maytenus robusta*; Gb *Gaylussacia brasiliensis*; Ea *Erythroxylum amplifolium*; Op *Ocotea pulchella*; Or *Ossaea retropila*; Cp *Cissampelos pareira*; Ru *Rapanea umbellata*; Mm *Myrcia multiflora*; Go *Guapira opposita*; Pn *Psychotria nuda*; Sb *Smilax brasiliensis*; Tb *Ternstroemia brasiliensis*

1 E. Cazetta, unpublished data (15 h of observation); 2 E. Cazetta, personal observation; 3 Francisco and Galetti (2001) (60 h of observation); 4 Francisco and Galetti (2002) (72 h of observation); 5 Manhães (2003); 6 Pizo et al. (2002); 7 M.A. Pizo, personal communication; 8 Schrerer et al. (2007); 9 Staggemeir and Guaraldo, personal communication

* Observations for species of the same genus

glucose, fructose, and sucrose by gas chromatography–mass spectrometer (modified from Pooter and Villar 1997). We used the mean gross energy equivalents of protein (17.2 KJ/g), lipid (38.9 KJ/g), and sugars (17.2 KJ/g, Karlsson 1972) to determine the energetic value of dried fruit pulp for each species.

To evaluate plant defenses, we determined the contents of phenols and condensed tannins in fruits. We used these compounds for two reasons. First, they are the classical example of quantitative defenses, which deter herbivores (Janzen 1974; Harborne 1979, 1980; Laks 1989). There are numerous examples of their general antifungal, antibacterial, and antiherbivore activities (Davidson and Juneja 1990; Scalbert 1991; Cipollini and Stiles 1993; Panizzi et al. 2002). Moreover, they also deter vertebrate herbivores and frugivores (Provenza et al. 1990; Clark et al. 1991; Guglielmo et al. 1996; Schaefer et al. 2003). Second, phenols and tannins are among the most widespread secondary compounds in ripe fruits (van Buren 1970; Herrera 1982; Cipollini and Stiles 1992b; Foley et al. 1995). In a broad interspecific comparison such as our where species originate from different plant families, it is crucial to use such widespread compounds that occur in fleshy fruits. A potential caveat is that we use the total contents of phenols and tannins as a proxy of biological activity, while some compounds might not have effects on fruit consumers. As this problem reduces the likelihood of finding a consistent pattern between the contents of secondary compounds and fruit consumption of vertebrates and pathogens, we consider our approach conservative. We extracted these compounds by the Price and Butler (1977) method in butanol and methanol extracts (see Schaefer et al. 2003). The contents of these compounds were analyzed with photometric measurements. For each fruit sample, we ran three replicates and used the mean of the three values for statistical analyses.

Data analysis

In a seminal study on fruit characteristics in 910 species, Jordano (1995) found that chemical and morphological fruit traits were decoupled. We therefore used two principal component analyses (PCA) with Varimax rotation to evaluate major independent trends in the variation of morphological and chemical fruit traits, similar to previous studies (Schaefer et al. 2003). Separate PCA were used because combining suites of traits having low correlation between each other results in PC analyses that do not capture significant variation in these traits. We included energy in these analyses, even though it is a compound variable, because birds might forage on total energy contents rather than separately on the contents of lipids, proteins and sugar.

The mean persistence time for all species of bagged and open was compared using Wilcoxon signed rank test. The

mean persistence time of bagged fruits was compared with the mean persistence time of open fruits across all species with a paired *t* test. The rate at which ripe fruits disappeared was estimated using Kaplan–Meier survival estimates (Willson and Whelan 1993; McCarty et al. 2002). Survival analysis is used when the response variable of interest is a time period until an event occurs. This type of analysis allows the inclusion of events that did not occur, because of the end of an experiment or individual loss (i.e., right-censored data). We calculated one rate for bagged fruits and one rate for open fruits.

We performed different multiple regressions with the two survival rates as response variables. We obtained the same results with forward and backward stepwise procedures. All survival rates were log transformed prior to analysis. We used factor scores of all the chemical principal components and the first morphological principal components as independent variables. We excluded the second and third morphological PCs because we were particularly interested in the effects of chemical variables based on our study questions. Moreover, previous studies reported that chemical characters are more important than morphological ones (Schaefer et al. 2003; Tang et al. 2005) and the first morphological PC accounts for most of morphological variance allowing us to correct for fruit morphology.

Results

Fruit traits

The PCA on morphological traits revealed that the first three components accounted for 87.45% of total variance. Fruit size (diameter and length) was correlated with fruit mass (Table 2). Variation in these traits represented the first principal component (PCM1) explaining 41.75% of the overall variation. The second principal component (PCM 2) is associated with seed size and mass. Fruits with low pulp to seed ratio and high relative yield show high values on the third principal component (PCM3) (Table 3).

The PCA for nutritional traits identified three components that accounted for 84% of total variance. Energy was positively correlated with lipids and negatively correlated with water (Table 4). Variation in these traits represented the first principal component (PCQ1), explaining 39.32% of the variation. Fruits with low contents of phenols and condensed tannins scored high on the second principal component (PCQ2), explaining 30.52% of the overall variation. Fruits with low protein contents scored high on the third principal component (PCQ3) (Table 5).

The first chemical principal component was correlated with the second and third morphological factors ($r_s = 0.54$, $P = 0.04$; $r_s = 0.68$, $P = 0.007$; respectively). Hence, fruits

Table 2 Spearman correlation of morphological fruit traits

	R _Y	Pulp/seed	No. seeds	1 seed mass	Seed length	Seed diam.	Fruit mass	Fruit length
Fruit diameter	−0.35	0.30	0.58*	0.39	−0.07	0.10	0.89*	0.59*
Fruit length	0.32	−0.21	0.28	0.64*	0.34	0.16	0.49**	
Fruit mass	−0.21	0.42	0.52	0.22	−0.20	−0.09		
Seed diameter	−0.13	−0.05	−0.50	0.73**	−0.30			
Seed length	0.25	−0.22	−0.30	0.56*				
1 seed mass	−0.11	0.01	−0.28					
No. seeds	−0.16	0.22						
Pulp/seed	−0.57*							

* $P < 0.01$, ** $P < 0.001$ **Table 3** Principal component analysis of morphological traits (only factor loadings >0.5 are shown)

Morphological traits	PCM1	PCM2	PCM3
Fruit diameter	0.95		
Fruit length	0.92		
Fruit mass	0.89		
1 seed mass	0.51	0.82	
Seed number		− 0.66	0.54
Seed diameter		0.98	
Pulp/seed	0.94		− 0.81
R _Y	−0.58		0.74
Eigenvalue	3.34	2.28	1.37
% variance explained	41.75	28.51	17.18
Cumulative variance	41.75	70.26	87.45

Major relationships (factor loadings > 0.6) are typed in boldface

Table 4 Spearman correlation of nutritional fruit traits

	Tannins	Phenol	Water	Energy	Sugar	Protein
Lipids	−0.03	0.12	−0.46	0.93**	−0.3	−0.1
Proteins	0.14	0.44	−0.22	0.12	−0.1	
Sugar	0.10	−0.19	0.35	−0.30		
Energy	0.05	0.16	−0.56*			
Water	0.18	0.16				
Phenol	0.45					

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

with high lipids and energy contents had higher seed mass, seed diameter and relative yield and lower pulp/seed ratio. The second chemical principal component was negatively correlated with the second morphological factor ($r_s = -0.76$, $P = 0.001$). Fruits with high phenols and tannins contents had higher seed mass and seed diameter.

Fruit fates

The mean fruit removal efficiency was 57.29% (range 26.6–83.5%). Fruit removal efficiency of four species was around 80% and *Ossaea retropila* (Melastomataceae) and

Table 5 Principal component analysis of nutritional traits (only factor loadings >0.5 are shown)

Chemical traits	PCQ1	PCQ2	PCQ3
Lipids	0.95		
Proteins			− 0.97
Sugar	−0.51		
Energy	0.95		
Water	− 0.80		
Phenols		− 0.78	−0.52
Tannins		− 0.94	
Eigenvalue	2.75	2.13	0.99
% variance explained	39.32	30.52	14.17
Cumulative variance	39.32	68.85	84.03

Major relationships (factor loadings > 0.6) are typed in boldface

Myrcia multiflora (Myrtaceae) had the lowest removal efficiency (26.5 and 32.9%, respectively) (Table 5). There was no correlation between removal efficiency and crop size ($r_s = 0.17$, $P = 0.09$), distance to other fruiting trees (D1: $r_s = 0.06$, $P = 0.57$; D2: $r_s = 0.03$, $P = 0.86$), and plant height ($r_s = -0.08$, $P = 0.40$). We likewise found no influence of these variables if they were included as co-variables in General Linear Models (GLM) with fruit removal of bagged and unprotected fruits as dependent variable.

The mean persistence time of bagged and open fruits across all species was different ($t = 11.14$, $P < 0.0001$). Fruits protected against frugivores persist longer than fruits open to them (Fig. 1; Table 6). We also found interspecific differences in the exposure time of ripe fruits in both treatments: when seed dispersers were excluded (Wilcoxon, $P < 0.0001$) or included (Wilcoxon, $P < 0.0001$).

Influence of fruit traits on persistence and removal

Fruit characteristics influenced persistence time of bagged and open fruits. Fruits with high lipid and energy contents persist for a short period of time, whereas fruits with high phenol and tannin contents persist for long periods in both treatments. Fruit size influences the persistence time only of

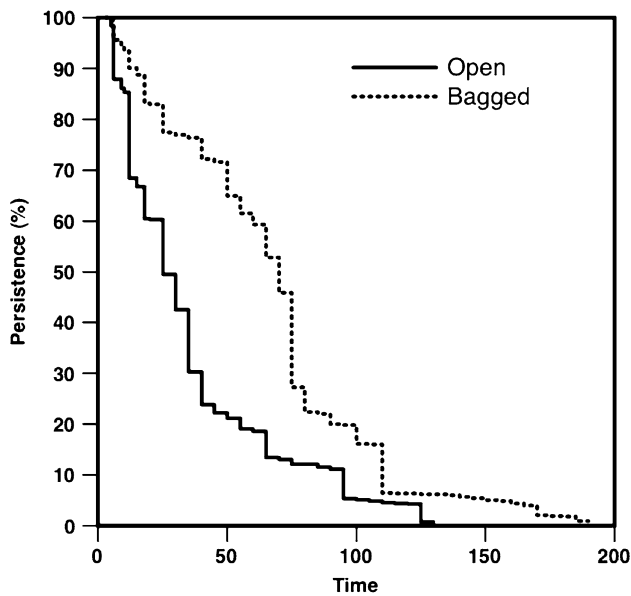


Fig. 1 Fruit persistence in the open and bagged treatment of all species evaluated, based on Kaplan–Meier survival estimates

bagged fruits so that larger fruits persisted for a shorter period than small fruits when vertebrates were excluded (Table 7).

Discussion

This study evaluated for the first time the influence of nutrients and secondary compounds on fruit consumption by vertebrate seed dispersers and pathogens. We found that fruit species that are quickly removed by both, vertebrate seed dispersers and pathogens, are nutrient-rich and contain lower amounts of phenols and condensed tannins. In con-

trast, fruit species that persisted longer, both when vertebrate consumers were excluded or when they had access to the fruits, are nutrient-poor and contain higher amounts of these secondary compounds. Thus, our results corroborate the removal rate model of the defense-trade off hypothesis.

Our results showed that fruits with high lipid and energy contents are quickly removed in all treatments. Frugivores may forage for fruits that best satisfy their nutritional demands, as stated by the nutrient content hypothesis (Manasse and Howe 1983; Denslow 1987; Levey 1987). However, pathogens also prefer nutrient-rich fruits and thus select against them. Our result suggests that we can predict how fruiting plants, seed dispersers, and pathogens interact simultaneously with the other players in the evolutionary triad (Tsahar et al. 2002; Tewksbury 2002). We suggest that according to the relative speed of fruit removal, plant species are located on different positions along the continuum of optimal defenses against antagonists and optimal attraction to seed dispersers.

The defense trade-off hypothesis builds on the assumption that secondary compounds mediate fruit persistence by their antimicrobial and antifungal effects (Jones and Wheelwright 1987; Cipollini and Stiles 1992b). We corroborate this notion because fruits with high phenols and tannins persisted for long time periods when vertebrates were excluded. We admit that natural abscission may have biased our estimates of the attack rate of antagonists in our experiment. This might be true particularly for bigger fruits, which persisted for shorter periods when vertebrates were excluded. However, the overall negative relationship between secondary compounds and fruit persistence time is unlikely to be explained primarily by natural fruit abscission. We therefore believe that the effects of natural abscission do not change the main result of this study.

Table 6 Mean crop size, removal efficiency, and the time that fruits remain available (persistence in days—mean ± S.E.) for bagged (B) and open fruits (O)

Species	Crop size	Removal efficiency (%)	B	O
<i>Cissampelos pareira</i>	740	45.45	68.82 ± 0.3	59.66 ± 0.57
<i>Ossaea retropila</i>	211	26.56	39.64 ± 0.13	33.19 ± 0.27
<i>Erythroxylum amplifolium</i>	18	50	11.24 ± 0.37	10.05 ± 0.61
<i>Gaylussacia brasiliensis</i>	107	56.6	61.75 ± 0.88	51.65 ± 0.70
<i>Geonoma schottiana</i>	300	48.5	150	119.26 ± 0.12
<i>Guapira opposita</i>	250	75	26.73 ± 0.07	16.79 ± 0.22
<i>Maytenus robusta</i>	977	32.91	69.42 ± 0.93	62.62 ± 0.69
<i>Myrcia multiflora</i>	102	80.7	57.51 ± 0.55	42.35 ± 0.41
<i>Ocotea pulchella</i>	148	50	33.18 ± 0.13	29.93 ± 0.004
<i>Psychotria nuda</i>	28	79.26	26.85 ± 0.98	18.96 ± 0.46
<i>Rapanea umbellata</i>	3733	83.47	90	78.74 ± 0.23
<i>Schinus terebinthifolius</i>	8373	50	55	37.49 ± 0.09
<i>Smilax brasiliensis</i>	458	44.6	172.72 ± 0.32	126.85 ± 0.57
<i>Ternstroemia brasiliensis</i>	212	79	14.96 ± 0.02	11.93 ± 0.03

Table 7 Multiple regression for the persistence rate of bagged and open fruits. Only significant independent variables are shown

Dependent variable	Source	Regression coefficient	SE	<i>T</i>	<i>P</i>	Standard regression coefficient	<i>R</i> ² (%)	<i>P</i>
Bagged	PCM1	−0.1	0.051	−1.99	0.048	1.04	60	<0.0001
	PCQ1	−0.44	0.051	−8.82	<0.0001	20.42		
	PCQ2	−0.43	−0.43	−8.98	<0.0001	21.16		
Open	PCQ1	−0.37	0.055	−6.73	<0.0001	16.02	52	<0.0001
	PCQ2	−0.49	0.056	−8.87	<0.0001	27.81		

Avian frugivores also avoided fruits rich in phenols and tannins suggesting that the increase of fruit persistence comes at a cost owing to a concomitant decrease in the palatability of fruits to seed dispersers (Foley et al. 1995; Schaefer et al. 2003). While many other classes of secondary compounds contribute to plant defense, e.g., glycoalkaloids (Cipollini and Levey 1997a; Levey and Cipollini 1997; Wahaj et al. 1998), our results demonstrate that phenols and tannins predict both, fruit avoidance of vertebrates and persistence time of fruits in the absence of fruit removal by vertebrates. This pattern fits the conjecture that phenols and tannins constitute quantitative plant defense (van Buren 1970; Harborne 1979; Herrera 1982). The presence of phenols might further be correlated with that of other secondary compounds with strong defensive activity (e.g., compounds produced by the phenyl pathway Fineblum and Rausher 1997).

We found an inverse relationship between levels of nutrients and phenols and condensed tannins; plant species that were quickly removed by dispersers and pathogens contained more nutritional reward and lower amounts of secondary compounds. Although pathogens also preferentially attack fruits rich in nutrients, these fruits retain low levels of defense. This paradox is explicable because the removal rate of seed dispersers is higher than that by pathogens. Consequently, the most rewarding fruit species are attractive enough to seed dispersers to offset the detrimental effects of pathogens. The removal rate model thus explains the pulp composition of the plant species that we studied. Previous studies also supported the removal rate model, concluding that nutrients stimulated fruit removal by seed dispersers while secondary compounds deterred them (Cipollini and Stiles 1993; Cipollini and Levey 1997a; Schaefer et al. 2003).

While nutrient-rich fruits are quickly consumed by mutualists and thus are poorly defended, nutrient-poor fruits are neither preferred by vertebrate fruit consumers nor attacked quickly by pathogens. Because the probability of a ripe fruit being attacked by antagonists increases linearly with exposure time (Thompson and Willson 1978), long persisting fruits are highly defended by secondary compounds. We suggest that—over evolutionary time—these species improved their competitive ability by higher

investment in fruit defense. This strategy might be particularly important for plant species that depend on rare or unreliable seed-dispersers (Cipollini and Stiles 1992c; Tang et al. 2005). For dispersers that consume these fruits sporadically, the quantities of deterrent substances will not exceed critical levels. Deterrent compounds might thus not strongly affect the intrinsically low individual consumption rates inherent to this kind of fruits (Herrera 1982).

Although the nutritional value of the fruits and the digestive ability of birds play a major role in fruit choice (Martinez del Rio and Restrepo 1993), many studies on seed dispersers have demonstrated the importance of characteristics such as fruit abundance, crop size, neighborhood effect, fruit size, color, among others (Sargent 1990; Murphy 1994; Saracco et al. 2005, etc.). However, as far as we know, similar studies evaluating how these characteristics influence fruit consumption by pathogens have never been conducted. We evaluated some of these traits in the present study but found no influence on fruit removal by vertebrates and pathogens. Plant characteristics such as height and crop size and neighborhood effects also did not influence fruit removal by seed dispersers in our study.

In conclusion, we demonstrate that chemical characteristics primarily influence preferences and removal rate by a broad range of frugivores. We found that nutrient-rich fruits are simultaneously attractive to both, pathogens and vertebrates. In spite of the high attack rate of pathogens, these fruits are poorly defended by secondary compounds suggesting that the removal rate of seed dispersers is the primary factor controlling the level of fruit defense. Selection for defense is high for fruits with low nutrient quality that are less preferred and consequently removed slowly. Because these species are highly defended, they retain ripe fruits in an intact condition for a prolonged period, which in turn reduces attractiveness but might be advantageous for plants that depend on rare or unreliable dispersers.

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