ABSTRACT

The present study assessed the fruiting pattern, bird foraging behavior, and sugar content of ripe fruits of two sympatric species of Rubiaceae (*Psychotria brasiliensis* and *P. nuda*). This study was carried out in an Atlantic forest area on Ilha Grande, RJ, between August 1998 and July 1999. Fruit production occurred year round, with a peak of mature *P. brasiliensis* fruits in December 1998 and another of *P. nuda* in February of 1999. *Lipaugus lanioides* (Cotingidae), *Baryptengus ruficapillus* (Momotidae) and *Saltator similis* (Emberizidae) made the most frequent foraging visits to fruiting *P. brasiliensis*, so that *L. lanioides* and *B. ruficapillus* removed the fruits with sallying maneuvers while *S. similis* gleaned the fruits. *Lipaugus lanioides* was by far the most important consumer, and potentially the main disperser of *P. brasiliensis*. Birds of this genus are heavy frugivores in the tropical forests and are widely assumed to be important seed dispersers. The fruits were analyzed quantitatively and qualitatively in relation to the amounts of sucrose and starch. *Psychotria brasiliensis* (the visited species) showed the smallest quantity of sucrose and the highest amount of starch. These findings suggest that what may influence the birds’ choice of fruit is the proportion of starch in the *Psychotria* species studied here rather than the carbohydrate composition.

Keywords: *Psychotria*, fruits, biochemical analysis.

RESUMO

Aves potenciais dispersoras de *Psychotria* em uma área de Floresta Atlântica, Ilha Grande, RJ, Sudeste do Brasil: uma análise bioquímica dos frutos

Os objetivos deste trabalho foram monitorar o padrão de frutificação, o comportamento de forrageamento de aves e o conteúdo de açúcares em frutos maduros de duas espécies simpátricas de Rubiaceae (*Psychotria brasiliensis* e *P. nuda*). O estudo foi realizado em área de Floresta Atlântica, na Ilha Grande, RJ, entre agosto de 1998 e julho de 1999. A frutificação ocorreu durante todo o ano com um pico de frutos maduros em dezembro de 1998 para *P. brasiliensis* e outro em fevereiro de 1999 para *P. nuda*. *Lipaugus lanioides* (Cotingidae), *Baryptengus ruficapillus* (Momotidae) e *Saltator similis* (Emberizidae) foram os principais consumidores dos frutos de *P. brasiliensis*, sendo que *L. lanioides* e *B. ruficapillus* removeram os frutos adejando, enquanto *S. similis* os alcançou pousado nas plantas. *Lipaugus lanioides* foi o consumidor de frutos mais importante e potencialmente foi o principal dispersor das sementes de *P. brasiliensis*. As aves deste gênero estão entre os principais frugívoros especialistas de florestas tropicais e amplamente consideradas como importantes agentes dispersores de sementes. Os frutos foram analisados qualitativa e

*Potentia Bird Dispersers of Psychotria in a Area of Atlantic Forest on Ilha Grande, RJ, Southeastern Brazil: A Biochemical Analysis of the Fruits*

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(With 3 figures)
quantitativamente em relação às quantidades de açúcares livres e amido. A espécie visitada (*P. brasiliensis*) apresentou a menor quantidade de sacarose e a maior quantidade de amido. Os resultados sugerem que o consumo diferencial de frutos por espécies de aves não é influenciado pela composição de carboidratos, mas possivelmente pela proporção de amido nos frutos das espécies de *Psychotria* estudadas.

**Palavras-chave:** *Psychotria*, frutos, análise bioquímica.

**METHODOLOGY**

**Study area**

This study was carried out in an almost undisturbed Atlantic forest area on Ilha Grande (23° 10’ 57” S and 44° 12’ 54” W), municipality of Angra dos Reis, in southern Rio de Janeiro state. The altitude of the study area ranges from 140 m to 260 m, and the canopy is around 25 to 30 m. The climate is hot and humid with an annual average temperature of 23 ºC (19.6° - 25.7°), while rainfall is approximately 2200 mm (data provided by Fundação Instituto de Desenvolvimento Econômico e Social do Rio de Janeiro).

**Phenology**

Fruit production was recorded monthly, between August 1998 and July 1999, using a transection of 1 ha (1000 m x 10 m). The number of unripe and ripe fruits was counted in 19 individuals of *P. brasiliensis* and 24 individuals of *P. nuda* randomly selected in the transection. The ripe fruits were weighed (with an electronic scale to the nearest 0.0001 g) and measured (using calipers to the nearest 0.1 mm).
Observation of the birds

Visits by birds to each plant species (15 individuals per species) were monitored on four days for a total of 96 h between September and December 1998. Three or four plants were watched simultaneously each day. The bird species visiting the plants, the time, the number of fruits eaten and the foraging tactics were recorded, allocating an interval of five minutes to record the foraging tactics of each individual observed (Alves & Duarte, 1996). The classification of the foraging tactics was adapted from Remsen & Robson (1990) and Marini (1992), as follows: a) gleaning – the bird removes the fruits while perched on a branch close to the infructescence; and b) sallying – from a nearby perch the bird flies towards the infructescence, hovering or not in front of it, removing a fruit and usually returning to the same branch or another one (Figs. 1-3).

Comparative biochemical analysis of carbohydrates of P. brasiliensis and P. nuda fruits

Four mature fruits from four different individuals of each plant species were collected. These fruits were dehydrated in an oven at 60 °C and weighed every 15 min until the weight became constant. The dried fruits were ground and 10 mg of powder were extracted in 500 μL of 80% ethanol at 80 °C for 20 min. The purpose of this procedure was the extraction of soluble sugars. The mixture was centrifuged for two minutes at 13,000 g, and

Fig. 1 — Baryptengus ruficapillus displaying the foraging tactic of sallying when consuming fruits of Psychotria brasiliensis (see explanations of the behavior in the text).

Fig. 2 — Saltator similis displaying the foraging tactic of gleaning when consuming the fruits of Psychotria brasiliensis (see explanations of the behavior in the text).
the supernatant removed for soluble sugar analyses. This procedure was repeated thrice and the precipitate and supernatants were combined. The alcohol extracts were freeze-dried and dissolved in deionized water. The total sugars were determined by the phenol sulphuric acid method (Dubois et al., 1956) and the reducing sugars by the method of Somogyi-Nelson (Somogyi, 1945).

The monosaccharides were analyzed by High Performance Anion Exchange Chromatography (Dionex DX-500) using a CarboPac PA-1 separation column. Monosaccharides were separated isocratically in distilled water. Sugar was detected by Pulsed Amperometric Detection (PAD) using a post column base containing 500 mM NaOH. The detection of glucose, fructose and sucrose was performed by isocratic separation in 100 mM NaOH using the same aforementioned column. A minor contamination with other monosaccharides was detected but, being minimal, it did not influence the results of sucrose, glucose and fructose (Tiné et al., 2000).

The starch was analyzed by the following procedure: the pellet resulting from the above described extraction of soluble sugars was subjected to complete alcohol evaporation at ambient temperature for about 24 h, and 2.8 mL of distilled water and 0.1 mL of thermo-stable α-amylase (Megazyme) were added. After stirring, the material was incubated at 75 °C for 30 min, 0.1 mL of thermo-stable α-amylase was added and the mixture stirred for 30 min at 75 °C. One mL of 300 mM sodium acetate buffer pH 4.8 and 1 mL of amylloglucosidase (30 U/mL) in 100 mM sodium acetate buffer pH 4.8 were added. The mixture in the tube was stirred for a few minutes and then incubated at 50 °C for 30 min. The reaction was interrupted by the addition of 500 µL of 0.8 M perchloric acid, followed by stirring and centrifugation at 13,000 g for 3 min. 20 µL of the mixture was removed and placed in the wells of an ELISA plate. 150 µL of Glucose PAP Liquiform 1 (Labstet®) were added to each well and the plate was incubated at 30 °C for 15 min, with ELISA readings taken at 490 nm. The starch content was calculated as a percentage of the weight of the initial material (adapted from Santos et al., 2004).

**RESULTS AND DISCUSSION**

The fruits of *P. brasiliensis* and *P. nuda* are purple drupes with yellow and red calyces, respectively. The fruits of these species exhibited significant differences in size and weight (Table 1).

Fruit production occurred year round (see also Almeida & Alves, 2000), with a peak of ripe *P. brasiliensis* fruits in December 1998. In that month, 4 of the 19 sampled individuals bore mature fruits (total of 19 mature fruits). In *P. nuda*, the major fruit production was in February 1999.
Three bird species foraged on *P. brasiliensis* fruits. The most frequent visitor was *Lipaugus lanioides* (Contigidae, 63.6%, n = 11), followed by *Baryphthengus ruficapillus* (Momotidae, 27.3%, n = 11) and *Saltator similis* (Emberizidae, 9.1%, n = 11).

The foraging tactic used by *B. ruficapillus* was sallying. After alighting on the plant, the bird removed the fruit with a short descending flight and landed on the same plant, swallowing the whole fruit (Fig. 1). *Saltator similis* removed the fruits using the foraging tactic of gleaning. The individuals of this species landed on the plant and picked the fruits, crushing them with their beak turned up (Fig. 2). *Lipaugus lanioides* used the sally foraging tactic. After alighting on a perch next to the visited plant, individuals of this species generally collected the fruits during a flight, thereafter returning to the original perch or landing on a nearby one (Fig. 3). This bird species kept the fruit in its beak, knocking the calyx on a branch to remove it before eating the fruit.

*Lipaugus lanioides* was the main consumer of *P. brasiliensis* fruits and was the only species that removed the calyx from the fruits. This suggests *L. lanioides* is a frugivorous specialist, in contrast to the other two bird species. *Lipaugus lanioides* is a potentially important disperser of *P. brasiliensis* seed, particularly since it consumed more whole fruits and showed a specific behavior (removing calyx), also flying away from the parental plant soon after removing the fruits. Birds of this genus are among the heavy frugivorous species of the rain forests (Snow, 1981) and, together with the other members of the Cotingidae family, are important seed dispersers (Snow, 1971). *Baryphthengus ruficapillus* is a generalist species (Sick, 1997; Alves et al., 1999) which may contribute to the dispersal of *P. brasiliensis*. *Saltator similis* is known as an omnivorous species whose diet substantially includes seeds (Sick, 1997), and it must be a predator of *P. brasiliensis* seeds.

Due to the small number of ripe fruits per individual, even during the fruiting peaks (see above), a lower visit rate to *P. brasiliensis* bushes was expected. However, birds were not recorded foraging on *P. nuda* fruits, which were available in the same area during the same period and were subjected to the same observation effort as *P. brasiliensis*. It is interesting to note that although *P. brasiliensis* produces relatively few mature fruits, the most frequent consumer of its fruits was a species endangered both worldwide (Collar et al., 1992) and regionally (Alves et al., 2000). This species was not trapped as frequently as were other frugivorous bird species (*e.g.*, *Chiroxiphia caudata* and *Mionectes rufiventris*) in the mist nets used in the study area (M. A. S. Alves, pers. obs.). However, these latter species were not recorded consuming *Psychotria* fruits. These findings suggest that, in the study area, *P. brasiliensis* may be an important resource for *L. lanioides*, reinforcing this bird’s potential as the main seed disperser of this plant.

An analysis was made of the soluble carbohydrate content and composition and the starch and phenolic compound content (not shown) in an attempt to associate the fruits’ chemical composition with the frequency of the birds’ visits to the plants in question. Table 2 shows the results of a comparative analysis of the monosaccharides in *P. brasiliensis* and *P. nuda* fruits. The main components of the soluble sugars fraction are glucose and fructose. However, other monosaccharides such as galactose, fucose and mannose were also detected in minor

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**TABLE 1**

*Averages and standard deviations of length, diameter and weight of the fruits of *Psychotria brasiliensis* and *P. nuda*, in an area of Atlantic forest, Ilha Grande, RJ.*

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Species</th>
<th>t²</th>
<th>p*</th>
<th>df⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. brasiliensis</em> (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm)</td>
<td>11.1 (± 1.0)</td>
<td>4.024</td>
<td>0.0001</td>
<td>51</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>11.8 (± 1.2)</td>
<td>6.834</td>
<td>0.0001</td>
<td>51</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.864 (± 0.232)</td>
<td>7.136</td>
<td>0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td><em>P. nuda</em> (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm)</td>
<td>9.7 (± 1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>9.7 (± 1.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>0.497 (± 0.118)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* result of Student t-test
* value of p
⁺ degrees of freedom

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(4 out of 24 individuals of the sample, with a total of 12 mature fruits).

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proportions. The latter compounds are probably related to the fruit maturation process, during which degradation of cell wall polysaccharides occurs. Glucose and fructose probably derive from sucrose metabolism, possibly resulting in the mobilization of compounds stored in the fruits, as well as transport of photoassimilates. As Table 2 indicates, *P. brasiliensis* and *P. nuda* fruits showed similar proportions of glucose and fructose. However, *P. brasiliensis* fruits contained twice as much fucose and four times less rhamnose than *P. nuda* fruits. Fucose, rhamnose and galactose are probably related with the cell wall metabolism and are generally used as indicators of the ripening stage of fruit (Giovannoni, 2001). In the fruits of dicots, except for glucose and fructose, galactose may be a good indicator of the ripening stages of fruits. A rising level of this monosaccharide may indicate that cell walls are being degraded, suggesting the occurrence of senescence, which is directly correlated with fruit ripening (Gross & Wallner, 1979). The proportion of carbohydrates in the fruits of both species indicated they were in the same ripening stage, since the proportions of monosaccharides of the cell wall were minimal and the levels of galactose in the two species were very similar.

The use of another program for separating sugars in HPAEC enabled us to view the glucose, fructose and sucrose simultaneously in the same chromatogram. As Table 3 indicates, the two species showed similar fructose – glucose ratios, but the proportion of sucrose was 2-fold higher in *P. nuda* than in *P. brasiliensis*. In addition, 0.54% of the dry weight of the *P. brasiliensis* fruits was starch, while that of *P. nuda* was only 0.13%, an inverse pattern to that of sucrose.

Together with the finding that the two species contained similar levels of phenolic compounds (data not shown), the results of the carbohydrate analyses suggested that both sugar compositions and their proportions were also similar in these species. However, the higher proportion of sucrose observed in *P. nuda* apparently was unrelated to the behavior of birds, since none of them consumed *P. nuda* fruits. These results suggest that the carbohydrate composition of fruits did not influence the birds’ choice of fruit. However, the higher proportion of starch in *P. brasiliensis* fruits may have influenced their choice. On the other hand, attractive or repulsive compounds not included in this study may also explain the contrasting use of fruits by the two species.

The relatively low seasonality of environments such as the Atlantic forest may explain the availability of ripe fruits for different species in the community (Talora & Morellato, 2000) throughout the year. This means food resources are continually

**Table 2**

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th><em>Psychotria brasiliensis</em></th>
<th><em>Psychotria nuda</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>52.56</td>
<td>52.32</td>
</tr>
<tr>
<td>Fructose</td>
<td>45.74</td>
<td>46.30</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.63</td>
<td>0.56</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.53</td>
<td>0.41</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.51</td>
<td>0.25</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.04</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Table 3**

Ratio (%) of the main nonstructural carbohydrates (glucose, fructose and sucrose) in mature fruits of *Psychotria brasiliensis* and *P. nuda* from an area of Atlantic forest, Ilha Grande, RJ.

<table>
<thead>
<tr>
<th>Species</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td><em>Psychotria brasiliensis</em></td>
<td>52.3</td>
</tr>
<tr>
<td><em>Psychotria nuda</em></td>
<td>47.5</td>
</tr>
</tbody>
</table>
available for frugivores. However, the continuous fructification allied with the low number of ripe fruits per plant, as observed in the Psychotria species studied (Almeida & Alves, 2000), seems to increase the potential for seed dispersion over a long period of the year, possibly increasing the adaptive efficiency of these species.

Acknowledgments — This article describes some of the results of studies carried out for the “Southeastern Brazilian Ecosystems Ecology, Conservation and Management Program” and the “Southeastern Brazilian Vertebrate Ecology Project” (Laboratory of Vertebrate Ecology), both conducted under the auspices of the Departamento de Ecologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro (UERJ). We thank Dr. Marcia Regina Braga, from the Seção de Fisiologia e Bioquímica de Plantas of the Instituto de Botânica, SP for the phenolic analyses of the Psychotria fruits. The Centro de Estudos Ambientais e Desenvolvimento Sustentável (CEADS) at UERJ’s Regional Campus on Ilha Grande, RJ, and the Sub-Reitoria de Pós-Graduação e Pesquisa (SR-2/UERJ) provided support and facilities on Ilha Grande during the study. We are also indebted to an anonymous referee for his critical review of the manuscript, as well as Raquel V. Marques for the sketches of bird foraging tactics, Brunnno A. N. Ribeiro for the first English draft, Conselno Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq, Brazil) for supporting the study and a research grant for M. A. S. Alves (nº 302718/2003-6), and CAPES for supporting E. M. Almeida with a Ph. D fellowship. The carbohydrate analysis was possible thanks to the financial support of the aril of Trichilia cuneata, a bird-dispersed fruit. Biotropica, 15: 26-31.


