Germination in Seed Species Ingested by Opossums: Implications for Seed Dispersal and Forest Conservation

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ABSTRACT

Seed germination in plant species consumed by opossums, genus Didelphis, was investigated in southern Brazil, in order to improve knowledge of the strategies of zoochorous plants in the Neotropics. Seeds were obtained from opossum feces. Thirteen of the most frequent species in the diet of local opossums were tested for germination rates and germination responses under different qualities (red/far red ratio) and different intensities of light. Most seeds from feces germinated similarly to the control groups, except for seeds of Rubus rosifolius, which appeared to depend on gut passage. Other experiments revealed that most seeds in the opossums' diet were of pioneer species, with most germination occurring during favorable humid conditions in the rainy season. A few species showed negative photoblastism, or no dormancy pattern. Small mammals are suggested as possible tools for area recuperation programs, through seed dispersal of many pioneer and some shade-tolerant plants, under suitable management.

Key words: Seed germination, light quality, dormancy, marsupials

INTRODUCTION

Angiosperm seeds generally show some type of dormancy, as a result of adaptation to environment in space and time. In nature, some seeds are adapted to germinate under light, others in its absence, by scarification, in decreasing or optimal temperatures, or through other strategies (Gómes-Pompa and Vázquez-Yanes, 1981; Metivier, 1985; Ferraz-Grande and Takaki, 2001). Many seeds and sprouts of Neotropical plant species germinate in the beginning of the wet season to assure full development during a period of surplus water (Garwood, 1983; Morellato et al., 2000). In many tropical species, light is the main cue to break dormancy and to begin development, especially in pioneer and secondary plants such as species of Piper and Cecropia (Gómes-Pompa and Vázquez-Yanes, 1981; Vázquez-Yanes and Orozco-Segovia, 1986; Orozco-Segovia et al., 1993; Vázquez-Yanes et al., 1996). The seed scarification that occurs in the digestive tract of animals is also important because, in addition to usually promoting germination (which is species-dependent), it is also related to transport of seeds away from the parent plant (Janzen, 1980; Reis and Guillaumet, 1983; Bodmer, 1991; Passos, 1997). However, under temperate conditions, seeds tend to show different germination strategies (Gómes-Pompa and Vázquez-Yanes, 1981), such
as the need to undergo the low temperatures of winter in order to germinate (Metivier, 1985).

Plants must be dispersed to suitable germination sites (Jordano, 1992; Schupp 1993). Among dispersal syndromes, those mediated by the wind and animals are prominent (Howe and Smallwood, 1982; Morellato et al., 2000). The most common animal syndromes are ornithochory by birds and chiropterochory by bats (Janzen, 1980; van der Pijl, 1982; Gorchov et al., 1993). There is no recognizable dispersal syndrome allocated to small, nocturnal, nonvolant mammals.

Didelphid marsupials are small mammals that are common in dense, cloud, and mixed Neotropical forests (Emmons and Feer, 1997). Didelphids seem to perform an important, but not well quantified, role in seed dispersal in the neotropics (Charles-Dominique et al., 1981; Atramentowicz 1988; Medellin, 1994; Cáceres et al., 1999; Carvalho et al., 1999). Nonetheless, little is known of their role in forest regeneration and maintenance. The role of Neotropical vertebrate frugivores in seed dispersal is usually studied in birds, bats and large mammals (e.g., Gorchov et al., 1993; Galleti and Aleixo, 1998; Da Silva and Tabarelli, 2000). In this context, the present study examined the germination strategies of seed species whose fruits are often consumed by marsupials of the genus *Didelphis*, in order to relate these strategies to seed dispersal and forest management in disturbed areas.

**METHODS**

**Study area**

The study was carried out in a small forest fragment (5 ha) in Curitiba, state of Paraná (25º 25’S and 49º 18’W, 940 m a.s.l.). The fragment is located in a suburban area belonging to the Companhia Paranaense de Energia (COPEL), and is surrounded by residences, buildings, roads and barren lands. The area was covered by disturbed and secondary mixed ombrophyll forest, with an unpolluted creek in its interior. Some of the main tree species were *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae), *Podocarpus lambertii* Klotzsch (Podocarpaceae), *Jasminum puberulum* Cham. (Bignoniaceae), *Ocotea puberula* Nees (Lauraceae), *Casearia sylvestris* Sw. (Flacourtiaceae), *Zanthoxylum rhoifolium* Lam. (Rutaceae), *Solanum sanctaeccatharinae* and *Solanum granulosus-leprosum* (Solanaceae). Common shrubs were *Piper gaudichaudianum* (Lauraceae) and *Rudgea jasminioides* Müell. Arg. (Rubiaceae); the lianas *Passiflora actinodiscata* Hooker (Passifloraceae) and *Pithecoctenium crucigerum* (L.) A.H. Gentry (Bignoniaceae) were also common. The mean annual temperature of the region is 17ºC. The annual rainfall of 1600 to 2000 mm is concentrated mainly in the warm season, September through March (Maack, 1981) (Fig. 1).

![Figure 1](image_url) - Monthly variation of rainfall (in mm) in the study area during the years when opossums were captured (1995-96: squares), and monthly means from 1979 to 1994 (circles)

**Trapping for feces and seeds**

Animals used for this study were *Didelphis albiventris* Lund, 1840 and *D. aurita* Wied-Neuwied, 1826 (Mammalia, Didelphidae), weighing usually 1 to 2 kg. To capture them, 30 live traps (~ 40x20x20 cm) were set in an irregular grid pattern (~ 40 m apart from each other) in the study area. Traps were set one night per week, for
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one year, from February 1995 through January 1996. Bait was a mixture of banana, peanut butter and cod liver oil. Additional data on *D. albiventris* feces and fruits were obtained in another area 8 km distant, with the same forest coverage and disturbance level.

Feces obtained from captured animals were searched for seeds, which were washed on a 1-mm mesh screen and analyzed under a stereomicroscope.

**Effects of gut passage on seeds**

Seeds found in feces were tested for the germination in closed Petri dishes containing moist paper. Results from individual or groups of seeds affected by fungi or excessively dried during the experiment were excluded from the analyses. To test the effect of gut passage on seeds, germination tests were also carried out with seeds of ripe fruits collected directly from parent plants (control treatment) in the study area, from 1997 through 1999. All seeds, from both treatments of feces and fruits, were placed for germination in a natural environment under shrubs, resembling a gap in the first successional stage of regeneration (*capoeira*).

**Germination under different light intensities and qualities**

Two groups of seeds (all from fruits) were treated separately under different light intensities to simulate environmental conditions, and were placed in a greenhouse. The first group was placed in a shaded site, out of direct sunlight, averaging 700 lux. The second group was placed in a culture chamber covered with 2 mm mesh, which decreased the sunlight by ~70% (10,000 lux). Daily variations of temperature and relative humidity were recorded in two periods of the year (June-July 1999 and January-February 2000), for 10-day periods, in order to monitor seasonal effects. These ranged from 16.4º to 21.3ºC and 84.1 to 86.7% on average for the chamber at light condition (respectively).

To determine what seed species were affected differentially by light quality for germination, the red/far red ratio test was used in the laboratory under ambient temperature. The red wavelength (660 nm) is more abundant in white light, as it reaches large forest gaps; whereas the far red wavelength (730 nm) is more abundant in the forest interior. The red wavelength is the cue for activation of germination in positive photoblastic seeds (Vázquez-Yanes and Orozco-Segovia, 1986).

Species with low germination frequencies were tested for negative photoblastic property by placing groups of seeds buried 1 cm deep in glass containers. Seeds were placed at the soil surface in adjacent glasses as a control. The maximum germination time (estimated time of dormancy) was recorded for those groups with more than 20 seeds germinated (for all treatments). Even if most seeds germinated in an experimental group, or even if none germinated in a short period of time, their seeds were monitored for 1 to 2 years.

**Statistic analyses**

The different comparisons among treatments were tested by using the proportion test ($p_1$ and $p_2$) based on sample sizes ($N$) and on the value of Student’s $t$ obtained (Statistica, 1993).

**RESULTS**

**Seed germination**

In total, 28 seed species were found in the feces of opossums (see details in Cáceres and Monteiro-Filho, 2001; and Cáceres, 2002). Only ~ 50% of the species were treated here (Table 1), because a part of the fruit species was not possible to collect in the field. All seeds were small in size (< 15 mm), and showed moderate to high germination frequencies.

Most of the seeds that passed through opossum guts did not show significant differences from the control groups. However, *Rubus rosifolius* germinated at significantly higher frequencies than the controls ($P < 0.01$; Table 1). Because they germinated fully under both shaded and lighted conditions, *Piper gaudichaudianum* and *Leandra australis* differed from the rest of the species, which germinated significantly only under light and at higher levels. Seeds of *Rubus rosifolius* and *Passiflora actinia* did not germinate well in either light or shade (Table 1).

As a result of the red/far red ratio test, 38% of all species responded to the test, showing positive photoblastism. *P. gaudichaudianum* and *L. australis* showed no response to these treatments, germinating in both cases (Table 1). *Rubus rosifolius*, *Cyphomandra corymbiflora* and *Solanum granulosum-leprosum* did not germinate in any treatment, under different light qualities.
Only *Passiflora actinia* was negatively photoblastic, with low levels of germination in light, but showed a 60% germination rate \( N=20 \) when their seeds were buried (contrasting with no germination at the soil surface \( N=20 \)).

**Dormancy**

Most seed species consumed by marsupials showed dormancy (62%), whereas only 31% showed rapid germination (less than two months). The mean time of dormancy prior to germination varied from six to 12 months, depending on the species. *P. gaudichaudianum* always germinated rapidly (in a few weeks), independent of treatment, never requiring more than two months to germinate (Table 1).

Most species germinated after the winter solstice, coinciding with the onset of the rainy season or germinated quickly, still in the same rainy season, when they were dispersed (Fig. 2).

**DISCUSSION**

At first analysis, didelphid marsupials feed mainly on fruits with small, pioneer seeds, but there were seeds with other strategies than germinating under full light, such as the mechanisms of germinating rapidly after dispersal, or even negative photoblastism. General ecological studies of seed dispersal by mammals are carried out only, and rather exclusively, by using germination under light conditions (e.g., Motta-Júnior et al., 1994; Passos, 1997; Cáceres and Monteiro-Filho, 2000; Quadros and Monteiro-Filho, 2000). However, the low levels of germination seen in those experiments may be due to other germination strategies, rather than under full light (e.g., negative photoblastism).

In general, seeds in the opossums’ diet showed dormancy, at least those that were ingested and usually appeared in their feces.

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**Figure 2** - Main period of dormancy and germination of seeds in the diet of *Didelphis* in mixed ombrophyll forest patches in southern Brazil. Genera are *Cyphomandra*, *Leandra*, *Morus*, *Piper*, *Physalis*, *Psidium*, *Rubus*, *Solanum* and *Vassobia*, respectively. Bars above indicate the warmer and wetter season. Vertical line indicates the winter solstice. ↓ indicates the mean time of seed dispersal; horizontal lines indicate mean duration of dormancy; ↑ indicates the mean time of seed germination.
An unknown number of fruits with larger seeds (presumably over 1.5 cm in diameter) is usually rejected by *Didelphis* near parent plants (e.g., Charles-Dominique et al., 1981; Atramentowicz, 1988). Because the study area was a small, disturbed forest fragment, this last fact should have favored the appearance of a higher proportion of small, pioneer seeds in the opossum feces (Vieira and Izar, 1999). Disturbed or secondary forests usually show a higher proportion of small, pioneer seed species in non-disturbed forest (Cáceres and Monteiro-Filho, 2001). However, *Didelphis* spp. appear to ingest more shade-tolerant seed species in non-disturbed forest (Vieira and Izar, 1999). Disturbed or secondary forests usually show a higher proportion of small pioneer seeds, that is, plant species that germinate under full light (Gómes-Pompa and Vázquez-Yanes, 1981; Garwood 1983; Gandolfi et al., 1995). In southeastern Brazil, species of *Leandra* (Melastomataceae) and *Solanum* (Solanaceae), taxa consumed by opossums, compose a large part of the forest seed bank (Baider et al., 1999; Grombone-Guaratini et al., 2004). This places these marsupials as potential integrants of the respective seed dispersal communities (see also Ulh et al., 1988). Furthermore, *Cecropia*, a genus also consumed by opossums (Medellín, 1994; Grell et al., 1999) and which exhibits dormancy, is also abundant in Neotropical forest seed banks (Vázquez-Yanes et al., 1996; Grombone-Guaratini et al., 2004).

**Table 1 - Germination frequencies (in %) of seeds from fruits and feces; effects of light intensity (%) and of red/far red ratio in seed germination and duration of dormancy in seeds dispersed by two opossum species (*Didelphis albiventris* and *D. aurita*), in disturbed mixed ombrophyl forest in southern Brazil.**

<table>
<thead>
<tr>
<th>Fruit species</th>
<th>D. albiventris</th>
<th>D. aurita</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces N</td>
<td>Feces N</td>
<td>Fruits N</td>
</tr>
<tr>
<td><em>Cyphomandra corymbiflora</em> Sendin.</td>
<td>98 (41)</td>
<td>60 (65)</td>
<td>82 (50)</td>
</tr>
<tr>
<td><em>Leandra australis</em> (Cham.) Cogn.</td>
<td>100 (13)</td>
<td>87 (30)</td>
<td></td>
</tr>
<tr>
<td><em>Morus nigra</em> L.</td>
<td>65 (71)</td>
<td>75 (8)</td>
<td>68 (25)</td>
</tr>
<tr>
<td><em>Passiflora actinia</em> Hooker</td>
<td>15 (41)</td>
<td>13 (71)</td>
<td>0 (20)</td>
</tr>
<tr>
<td><em>Physalis pubescens</em> L.</td>
<td>87 (100)</td>
<td>86 (38)</td>
<td>79 (110)</td>
</tr>
<tr>
<td><em>Piper gaudichaudianum</em> Kunth</td>
<td>94 (50)</td>
<td>94 (65)</td>
<td></td>
</tr>
<tr>
<td><em>Psidium guajava</em> L.</td>
<td>82 (11)</td>
<td>71 (24)</td>
<td>92 (25)</td>
</tr>
<tr>
<td><em>Rabius rosifolius</em> Stokes</td>
<td>57 (113)</td>
<td>72 (25)</td>
<td></td>
</tr>
<tr>
<td><em>Solanum granuloso-soleprosum</em> Dun.</td>
<td>71 (07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cf. maioranthum</em> L.B.Sm. and Downs</td>
<td>40 (90) *</td>
<td>57 (56) *</td>
<td>88 (25)</td>
</tr>
<tr>
<td><em>S. sanctaecatharinae</em> Roem. and Schult.</td>
<td>78 (63)</td>
<td>79 (110)</td>
<td></td>
</tr>
<tr>
<td><em>Vassobia breviflora</em> (Sendtn.) Munz.</td>
<td>52 (31)</td>
<td>25 (20)</td>
<td></td>
</tr>
</tbody>
</table>

**Fruit species**

<table>
<thead>
<tr>
<th>Light and shadow effects</th>
<th>Proportion</th>
<th>Dormancy (in months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Shadow</td>
<td>Light/Far red</td>
</tr>
<tr>
<td><em>Cyphomandra corymbiflora</em> Sendin.</td>
<td>88 (65) *</td>
<td>13 (30)</td>
</tr>
<tr>
<td><em>Leandra australis</em> (Cham.) Cogn.</td>
<td>46 (90)</td>
<td>32 (60)</td>
</tr>
<tr>
<td><em>Morus nigra</em> L.</td>
<td>24 (25)</td>
<td>4 (25)</td>
</tr>
<tr>
<td><em>Passiflora actinia</em> Hooker</td>
<td>0 (20)</td>
<td>0 (20)</td>
</tr>
<tr>
<td><em>Physalis pubescens</em> L.</td>
<td>66 (35) *</td>
<td>13 (70)</td>
</tr>
<tr>
<td><em>Piper gaudichaudianum</em> Kunth</td>
<td>80 (35)</td>
<td>85 (60)</td>
</tr>
<tr>
<td><em>Psidium guajava</em> L.</td>
<td>91 (22) *</td>
<td>27 (22)</td>
</tr>
<tr>
<td><em>Rabius rosifolius</em> Stokes</td>
<td>6 (35)</td>
<td>6 (33)</td>
</tr>
<tr>
<td><em>Solanum granuloso-soleprosum</em> Dun.</td>
<td>67 (30) *</td>
<td>0 (30)</td>
</tr>
<tr>
<td><em>S. cf. maioranthum</em> L.B.Sm. and Downs</td>
<td>100 (40)</td>
<td>10.0 R** (25)</td>
</tr>
<tr>
<td><em>S. sanctaecatharinae</em> Dun.</td>
<td>84 (25) *</td>
<td>0 (25)</td>
</tr>
<tr>
<td><em>S. swartzianum</em> Roem. and Schult.</td>
<td>71 (35) *</td>
<td>0 (25)</td>
</tr>
</tbody>
</table>

\[ **P < 0.01; *P < 0.05 for Proportion Test between feces vs. control, light vs. shade, and red vs. far red (Statistica, 1993). N is the total number of seeds tested in each treatment (in parentheses). R indicates the highest proportion of germination in the treatment under Red light; F indicates the highest germination under Far Red light. The mean time of dormancy is related to seed lots where at least 20 seeds germinated. All seeds were taken directly from fruits, except when indicated. a – specific seed lot with N less than 20. b - indicates origin from feces. c – N indicated in ( ) is the total for each one of the two treatment (R or FR); most treatments (R: FR ratio) with total N ≥ 40 seeds were replicated once (e.g. 20+20 seeds for FR); treatments with total N > 70 seeds were replicated three times. (+) indicates no response to the red/far red treatment.\]
The fact that *P. gaudichaudianum* germinated under all treatments, including red/far red ratios, indicates the absence of dormancy in this species, similar to the strategy adopted by *Ficus* in the northern Neotropics (Vázquez-Yanes et al., 1996). However, other species of *Piper* exhibit different strategies, such as dormancy for several years in unfavorable situations (Orozco-Segovia et al., 1993; Vázquez-Yanes and Orozco-Segovia, 1993). Thus, *P. gaudichaudianum* must be dispersed rapidly after fruiting and during favorable times (wet season) for its successful establishment (e.g., favorable temperatures and ample water; Socolowski and Takaki, 2004), to increase the chances of seedling survival (e.g., Foster, 1982; Garwood, 1983). Accordingly, seed germination is important during the early rainy season in the Atlantic rainforest, even in a less seasonal biome (Morellato et al., 2000) such as a mixed forest like the study area. Furthermore, habitat requirements for the development of *P. gaudichaudianum* are most probably the forest interior and small forest gaps (personal observation), since its seeds do not germinate reasonably under full light (Cáceres and Monteiro-Filho, 2000).

Shade-tolerant species occurred at low frequencies in the diet of opossums, at least in this small fragment. This could be partly due to the invasion of pioneer plants from the matrix (open area) to the forest interior, or from the edge to the forest interior, as seen elsewhere (Gandolfi et al., 1995; Tabanez et al., 1997; Da Silva and Tabarelli, 2000). This historical invasion must have changed the proportions of shade and pioneer plants in the fragment. This change can be inferred from the higher floral richness of pioneer plants recorded in a similar, but larger, forest fragment located only 1 km from the study area (C. Kosera, V.A.O. Dittrich and S.M. Silva, unpublished data). Nonetheless, *Didelphis* spp. must contribute to dispersal of seeds from outside fragments to the interior, and vice-versa (e.g., Cáceres and Monteiro-Filho, 2001), because of their high rates of migration and dispersal among fragmented landscapes (Pires et al., 2002).

The increase of germination capacity after opossum gut passage, as indicated for *Rubus rosifolius*, was previously reported for other wild mammals such as bats (Reis and Guillaumet, 1983) and primates (Figueiredo, 1993; Passos, 1997). In these cases, only a small number of seed species, in regard to the complete spectrum of species, exhibited this strategy. This increase in the probability of seed germination is thought to make certain plants more dependent on their dispersal agents for reproductive success.

Most opossum-dispersed seeds appear to be transported only endozoochorically and chemical scarification does not usually affect their viability, neither increasing nor decreasing germination rates. Such seed types (small-sized ones) are thought to accept a wide array of dispersal agents (e.g., Bodmer, 1991; Jordano 1992). Furthermore, in *Didelphis*, seeds pass relatively rapid through the gut, within 4 h to 1 day, depending on the plant species (N.C. Cáceres, unpublished data). This is not harmful to seeds, as pointed out by Jordano (1992) in studies of passage times for seeds in vertebrate guts.

According to the main germination time of most species seen here (averaging from the end of the winter solstice to the beginning of the period of heavy rainfall), it could be inferred that the dormancy break was related to, and initiated by increasing humidity in the seed coat and embryo after consecutive rains and/or increasing day length (Garwood, 1983; Metivier, 1985). The importance of humidity, or water surplus to increase the probability of germination when a seed is dormant (Vázquez-Yanes and Orozco-Segovia, 1993; Socolowski and Takaki, 2004) corroborates this.

Examination of the role of didelphid marsupials in the dynamics of seed germination and dispersal in fragmented and undisturbed areas has provided interesting scenarios and their role needs to be better understood. In light of the data presented and discussed here (germination probabilities, differential germination processes, dormancy and rapid germination, movement pattern of opossums in disturbed areas, etc.), opossums could be also considered as management tools for forest regeneration.

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RESUMO

Com o objetivo de fornecer conhecimento adicional acerca das estratégias de germinação adotadas por espécies de plantas zocóricas da Região Neotropical, foram examinadas a dormência e germinação de sementes consumidas por marsupiais do gênero Didelphis. As sementes foram obtidas de fezes dos animais capturados em armadilhas, sendo então analisadas. As espécies mais frequentes na dieta dos gambás (N = 13) foram testadas quanto a sua viabilidade e possíveis mecanismos de quebra de dormência pela luz (condições de luz vs. sombra vs. escuro; e sob red/far red ratio). A maioria das espécies provenientes das fezes germinou similarmente ao grupo controle, exceto por Rubus rosifolius que parece depender da passagem pelo trato digestivo para a germinação. Outros experimentos mostraram que as espécies de plantas eram principalmente pioneiras, com quebra de dormência principalmente durante a estação mais úmida e favorável ao desenvolvimento. Uma menor proporção de espécies apresentou fotoblastismo negativo ou nenhum padrão de dormência. Os resultados mostram que estes pequenos mamíferos podem ser utilizados em programas de recuperação de áreas degradadas, e enfoque na dispersão de sementes de plantas pioneiras.

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