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Reproductive success of four species of *Eugenia* L. (Myrtaceae)

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RESUMO – (Successo reprodutivo de quatro espécies de *Eugenia* L. (Myrtaceae)). *Eugenia uniflora*, *E. punicifolia*, *E. neonitida* e *E. rotundifolia* são espécies perenes, geralmente de porte arbustivo, que ocorrem na restinga do Parque Municipal Natural de Grumari, região oeste do Município do Rio de Janeiro, Brasil. Possuem flores-pólen do tipo *Papaver*, hermafroditas, polistêmones e polinizadas por abelhas. O sistema reprodutivo de cada espécie foi avaliado e os resultados mostraram que apenas *E. uniflora* e *E. punicifolia* são auto-compatíveis. De cada espécie foi avaliado o sistema de reprodução, as taxas fruto/flor, semente/óvulo e semente/fruto, além das taxas de predação de frutos e sementes e identificação dos seus agentes predadores. Com estes dados foram obtidos a taxa de fecundidade e o sucesso reprodutivo total de cada espécie. O sucesso reprodutivo total das quatro espécies foi muito baixo. Assim, para que no final do processo reprodutivo houvesse uma única semente viável e livre do ataque de herbívoros foi necessária a produção de 312,5 flores de *E. uniflora*, 9.090,9 de *E. neonitida*, 11.111,1 de *E. punicifolia* e 19.230,8 de *E. rotundifolia*. Em síntese, o sucesso reprodutivo total nas quatro espécies foi influenciado pelo sistema reprodutivo, pela razão semente/óvulo e, principalmente, pelas elevadas taxas de predação de frutos e sementes. A floração em massa destas espécies pode minimizar sua baixa eficiência reprodutiva, garantindo assim, a manutenção de sua dinâmica populacional.

Palavras-chave: *Eugenia*, herbivoria de sementes, Myrtaceae, sistema de reprodução, sucesso reprodutivo

ABSTRACT – (Reproductive success of four species of *Eugenia* L. (Myrtaceae)). *Eugenia uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* are perennial species, usually shrubs, which occur in the *restinga* of the Grumari Natural Municipal Park, in western Rio de Janeiro, Brazil. They have Papaver-type pollen-flowers that are hermaphrodite, polystemonous and pollinated mainly by bees. An assessment of the breeding systems showed that only *E. uniflora* and *E. punicifolia* are self-compatible. The fruit/flower, seed/ovule and seed/fruit ratios were calculated for each species. Fruit and seed predators were identified and predation rates were estimated. Total reproductive success for each species in the *restinga* was extremely low. In order to have one viable seed free from herbivore attack at the end of the reproductive process, the species would need to produce 312.5 *E. uniflora*, 9090.9 *E. neonitida*, 11111.1 *E. punicifolia* and 19230.8 *E. rotundifolia* flowers. In short, the reproductive success of the four species is affected by pollination efficiency, low seed/ovule ratios and mainly, high predation rates. Mass flowering strategy in these species can minimize low reproductive efficiency, thus ensuring the maintenance of population dynamics.

Key word: breeding system, *Eugenia*, Myrtaceae, reproductive success, seed herbivory

Introduction

The reproductive success of a species is directly linked to its population dynamics, since species stability in a given environment is dependant mainly on the amount and on the quality of their offspring, which allows them to be stable in their environment (Wiens 1984; Wiens *et al.* 1987). During the reproductive process, not all flowers produce fruit nor do all ovules become seeds. The fruits that persist do not have their dispersal assured and their seed germination is not guaranteed. Limiting factors occur at each stage of the reproductive process, reducing its efficiency, as discussed by Darwin (1859) and many other authors. These factors include pollination efficiency, energy resource allocation for fruit and seed production, natural abortion rates, flower, fruit and seed predation, as well as germination capacity (Wiens 1984; Wiens *et al.* 1987; Charlesworth 1989; Beardsell 1993a; b; Burd 1994; Dogteron *et al.* 2000; Cunningham 2000; Silva *et al.* 2002).

Seed production is intrinsically important for species as well as for the ecosystem, since they form seed banks, in addition to being a nutritional resource for herbivores (Zamith & Scarano 2004). Annual seed production depends not only on biological factors, such as pollination and maternal resource allocation, but also on environmental factors, such as mean annual precipitation and habitat fragmentation (Koening & Knops 2000;

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Murren 2002). Plant population dynamics is also influenced by the breeding system, since genetic variability is directly related to self and cross fertilization rates (Bawa 1974; Motten & Antonovics 1992). Homogeneous populations show less versatility for important evolutionary adaptations than do heterogeneous populations (Grant 1971).

There are several factors responsible for the selective abortion of ovules and seeds at different stages of development (Wiens 1987; Charleswhorth 1989). The seed/ovule and the fruit/flower ratios are the main parameters for evaluating species fecundity and can be used to measure the degree of reproductive efficiency of a population (Cruden 1972). Flower, fruit and seed predation is also a highly significant limiting factor for reproductive success and has a direct influence on population recruitment (Wenny 2000; Cunnigham 2000; Silva *et al.* 2002; Mahoro 2003; Cardoso & Lomônaco 2003).

Studies focusing on reproductive success and consequent seed production are very common in commercial plants. Bezerra *et al.* (1997), for example, described the annual fruit production of *Eugenia uniflora* L. cultivars. However, studies in natural areas are scarce, especially in the *restinga* (Silva *et al.* 2002).

Eugenia, with nearly 1000 species, is one of the most representative genera of Myrtaceae (Merwe *et al.* 2005), subfamily Myrtoideae, which includes genera with fleshy fruits (Lughadha & Proença 1996). Myrtaceae is an ecologically important family in Brazil's Atlantic Rainforest (Mori *et al.* 1983) and represents the largest number of species in the Brazilian *restinga* (Araújo & Henriques 1984; Lemos *et al.* 2001; Assis *et al.* 2004). Important research on Myrtaceae reproductive biology has already been carried out in Brazil (Proença & Gibbs 1994; Torezan-Silingardi & Del-Claro 1998; Maués & Couturier 2002; Torezan-Silingardi & Oliveira 2004; Silva & Pinheiro 2007), in Africa (Wyk & Lowrey 1988) and in Australia (Beardsell *et al.* 1993b). There is also a review paper on the topic (Gressler *et al.* 2006)

This article is part of a broader study about the reproductive biology of *Eugenia uniflora* L., *E. punicifolia* (Kunth) DC., *E. neonitida* Sobral and *E. rotundifolia* Casar in the *restinga* of Grumari, on the west coast of the city of Rio de Janeiro, Brazil. The floral and pollination biology results of these species have already been reported by Silva & Pinheiro (2007). The general objective of this study was to determine which factors limit seed production in this species. We aimed: a) to determine fecundity rates (Cruden 1972), b) to evaluate the breeding system c) to evaluate pollination efficiency, d) to determine seed predation rate, e) and to determine the overall reproductive success of each species.

Material and methods

Study area – The *restinga* of Grumari is located in the western region of the city of Rio de Janeiro, Brazil, from latitude 43º31'00''-43º32'30'' and longitude 23º02'30''-23º03'10'', between the districts of Recreio dos Bandeirantes and Barra de Guaratiba. This *restinga* is part of the "Área de Proteção Ambiental (APA) de Grumari" (Grumari Environmental Protection Area), which has recently been transformed into the "Parque Municipal Natural de Grumari" (Municipal Decree # 20149 - July 2, 2001), bordered by the "Parque Estadual da Pedra Branca" and by the "APA da Prainha". In the Grumari *restinga*, the Myrtaceae family is represented by eight species from two genera, *Eugenia* being the most representative genus with seven species. *Eugenia uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* are found mainly in beach and open *restinga* scrub formations (A.M. Argôlo, unpublished data).

Breeding system – The following tests were carried out to evaluate the breeding system: 1) self-pollination (autogamy) - transfer of pollen from a flower to its own stigma; 2) cross-pollination (xenogamy) - transfer of pollen to the stigma of a flower in another plant population. These tests were conducted with previously bagged flowers one day before anthesis; 3) Control - flowers that were left untreated; 4) automatic self-pollination - calculated from the proportion of fruits formed from bagged buds. The pollen load on the stigma of open (naturally pollinated) and bagged (automatic self-pollinated) flowers was determined by quantitative analysis of the pollen grains on the stigma, using an optical microscope. In *E. uniflora* and *E. punicifolia* (self-compatibility species) we removed the style of 75 and 114 buds, respectively, to test for the occurrence of autonomous apomixis (Richards 1986).

To evaluate the significance between the percentages obtained from xenogamy and the control experiments, the comparison formula was used between two percentages, with a 5% significance level (Pagano & Gauvreau 2004). The values observed in "t" were then compared with tabulated theoretical "t" values.

Seed/ovule and fruit/flower ratios – The seed/ovule ratio was evaluated using the mean number of seeds per fruit and the mean number of ovules per ovary. The fruit/flower ratio was based on the number of fruits after natural pollination. Sampling varied according to the species. To compare the differences between mean seed and fruit mass, we used the two-mean comparison formula, with a level of significance of 5% (Pagano & Gauvreau 2004). The "t" values observed were compared with tabulated theoretical "t" values.

Fruit and seed predation – The insect parasites were collected from fruits on the plants as well as from the litter. The fruits and seeds were placed in Petri Dishes with *restinga* substratum and covered with tulle mesh. These pots were periodically wetted to avoid dehydration. The animals were then stored in vials with 70% alcohol and sent to specialists for identification. The litter in a 50×50 cm area under three plants of each species was colleted up to a depth of 10cm and the samples were then analyzed in the laboratory to quantify the total number of viable and predated seeds. This procedure was carried out one month after the end of each species fructification period.

Reproductive success – To estimate the fecundity rate, we followed Cruden's (1972) procedure, which is the product of two ratios (seed/ovule and fruit/flower). Total reproductive success was evaluated using 100 hypothetical flowers, after which we applied the reproductive ratios found in each species as follows: mean number of ovules for each species, fruit/flower ratio from natural pollination and the ratio of predated litter seeds. The final index was obtained by calculating the ratio between the number of viable seeds at the end of the process and the number of ovules produced in the 100 hypothetical flowers. The final value corresponds to the total seeds produced for each 100 flowers. We, therefore, estimated how many flowers are needed to produce a single seed using a simple rule of three.

Results and discussion

Reproductive biology – *Eugenia uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* have hermaphrodite, polystemonous, Papaver-type pollen-flowers and generalist characteristics. They are visited by a range of insects, including species of Hymenoptera, Coleoptera and Diptera. Considering the foraging behavior and intra flower movements of the visiting insects, bees are the main pollinators of these plants and of these *Apis mellifera* L. is the most frequent and abundant pollinator (Silva & Pinheiro 2007).

Breeding systems – *Eugenia neonitida* and *E. rotundifolia* did not produce fruit in hand self-pollination experiments, indicating self-incompatibility (Tab. 1). In these same experiments, *E. uniflora* and *E. punicifolia* produced fruit (34.48% and 10.34%, respectively), showing that they are self-compatible (Tab. 1). In *Eugenia* there are both self-compatible (Proença & Gibbs 1994; Gressler *et al*. 2006) and self-incompatible species (Sobrevila & Arroyo 1982; Wyk & Lowrey 1988; Gressler *et al*. 2006), indicating a diversity of breeding systems in this genus.

Considering the pollination mechanism and the mass flowering of the studied species (Silva & Pinheiro 2007), self-compatibility can be responsible for an increase in fruit-set by geitonogamy, also reported in other Myrtaceae species (Lughadha & Proença 1996; Schmidt-Adam *et al.* 2000; Torezan-Silingardi & Oliveira 2004; Gressler *et al*. 2006). However, in complete or partial self-incompatible species, geitonogamy can be a limiting factor for fruit and seed production (Proença & Gibbs 1994; Mahoro 2003).

The mass flowering strategy can increase fruit and seed production in self-compatible species, because the abundance of flowers keeps the floral visitors in the plant for extended periods, thus, promoting self-pollination (Beardsell *et al.* 1993a). Comparing the fruit-set by natural pollination in the four species studied, which are pollinated by the same pollinator group (Silva & Pinheiro 2007), we were unable to determine which reproductive system was the most effective, because *E. neonitida* (a self-incompatible species) had higher rates of natural fruit-set than those of the two self-compatible species (Tab. 1), whereas *E. rotundifolia* (self-incompatible) had the lowest fruit-set (3.6%).

Eugenia uniflora and *E. punicifolia* (self-compatible species) had higher fruit-set from cross-pollination treatment than from hand self-pollination in

Table 1. Reproduction System of *Eugenia uniflora* L., *E. neonitida* Sobral, *E. punicifolia* (Kunth) DC. and *E. rotundifolia* Casar. X% (N).

Species	Pollination treatment			
	Natural condition	Hand cross-pollination	Hand self-pollination	Automatic self-pollination
Eugenia uniflora	14.96% ^{A}(127)	55.88% $^{A'}$(34)	34.48%^{AA}(29)	18.00%^{AAA}(50)
Eugenia neonitida	17.17% ^{B}(64)	21.43% $^{B'}$(19)	0%(26)	0%(60)
Eugenia punicifolia	16.4% ^{C}(61)	27.78% $^{C'}$(18)	10.34%^{CC}(39)	5.25%^{CCC}(438)
Eugenia rotundifolia	3.6% ^{D}(56)	8.33% $^{D'}$(22)	0%(21)	0%(42)

Comparison between fruit production ratio in the control and in the cross-pollination experiments. SD- significantly different, NSD- not significantly different: A-A'- SD ($t_{obs}=5.0 > t_{teor}=1.98$); A'-AA – NSD ($t_{obs}=1.69 < t_{teor}=2.01$); AA – AAA – NSD ($t_{obs}=1.62 < t_{teor}=2.01$); B-B'- NSD ($t_{obs}=0.36 < t_{teor}=2.01$); C-C'- NSD ($t_{obs}=1.09 < t_{teor}=2.01$); C'- CC – SD ($t_{obs}=18 > t_{teor}=2.01$); CC – CCC – NSD ($t_{obs}=1.56 < t_{teor}=1.96$); D-D'- NSD ($t_{obs}=0.57 < t_{teor}=2.01$).

the control treatment, which indicates that these species are preferentially outbreeders. This finding was also reported for *E. dysenterica* (Proença & Gibbs 1994), and in other Myrtaceae species (Butcher *et al.* 1992; Beardsell *et al.* 1993a; Torezan-Silingardi & Del-Claro 1998, Schmidt-Adam *et al.* 2000; Gressler *et al.* 2006). Sedgley & Smith (1989) found that even self-compatible species can have pollen-tube competition among pollen grains from the same plant and from different ones, leading to the greater fruit-set success of cross-fertilization.

Our results agree with studies on genetic diversity in *E. dysenterica* (Telles *et al.* 2001; Zucchi *et al.* 2003) and in *E. uniflora* (Margis *et al.* 2002; Salgueiro *et al.* 2004). These authors report that there is a greater genetic variation within populations than between them, due to the high ratio of genetic drift observed. According to Hamrick *et al.* (1993), high inter-population diversity occurs in wind- or animal-dispersed allogamic species.

Self-fertilization, owing to automatic self-pollination, was observed only in *E. uniflora* and in *E. punicifolia*, with a total fruit production of 18.0% and 5.25%, respectively (Tab. 1). These values are not significantly different from the ratios obtained in the hand self–pollination experiments (*E. uniflora*, $t_{obs}=1.62 < t_{teor}=2.01$, *E. punicifolia*, $t_{obs} = 1.56 < t_{teor} = 1.96$), indicating that automatic autogamy can be a strategy for the shortage of effective pollinators (Levin 1972; Bawa & Webb 1984). Proença & Gibbs (1994) did not find a significant difference between automatic self-pollination and hand self-pollination in self-compatible Myrtaceae species.

Pollen deposits on the stigma in *E. uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* in automatic self-pollination was possible because at the moment of floral opening, the anthers were already dehiscent and their pollen grains could contact the stigma when the style completed its growth (Silva & Pinehiro 2007). This phenomenon is common in other Myrtaceae species (O'Brien & Calder 1993; Beardsell *et al.* 1993a; b; Proença & Gibbs 1994; Gressler *et al*. 2006). The number of pollen grains found on the stigma of previously bagged flowers was smaller than that found on the stigma of open flowers (Tab. 2). However, the amount of grains deposited on the stigma originating from automatic self-pollination pistils was sufficient for the fertilization process.

Automatic self-pollination can offer a selective advantage under limited pollinator availability; in other words, there is fruit-set even without pollination. According to Motten & Antonovics (1992) and Navarro & Guitián (2002), this mechanism can favor the reproductive efficiency of a species in a fragmented environment where pollinator availability is limited.

In *Eugenia neonitida* and in *E. rotundifolia* (self-incompatible species), automatic self-pollination can hinder cross-pollination pollen tube growth, owing to competition with the autogamic pollen tubes, thus reducing reproductive success (Proença & Gibbs 1994; Mahoro 2003).

In the experiments to confirm apomixis in *E. uniflora* and *E. punicifolia*, no styleless bud developed into fruit, indicating the need of pollination for an effective fruit-set. Lughada & Proença (1996) discuss the apomictic process in a number of Myrtaceae species, including *Eugenia*, based on the occurrence of poliembryony. However, embryological studies are needed to clarify this issue in *E. uniflora* and in *E. punicifolia*.

Seed/ovule ratio – *Eugenia uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* had a low seed/ovule ratio (Tab. 3). *E. uniflora* had the highest S/O ratio (7.6%), and the largest mean number of seeds per fruit (1.6). The number of seeds in this species varied from one to five. *E. punicifolia* had just one seed per fruit and an S/O ratio of only 3.5%. In a sample of 25 *E. rotundifolia* fruits, just one contained two seeds (mean = 1.08, and S/O ratio of 5.19%). *E. neonitida* had an S/O ratio of only 4.4% and 1.5 seeds per fruit (Tab. 3). These values are considered low, given that many species of nonrelated families have ovule and seed abortion rates between 10 and 20% of total ovule production (Ramirez 1998).

The number of seeds per fruit is influenced by several factors, such as the efficiency of the pollination mechanism, energy resource availability, predation risk and population size (Charlesworth 1989; Cunningham

Table 2. Pollen grains on the stigma surface of *Eugenia uniflora* L., *E. neonitida* Sobral, *E. punicifolia* (Kunth) DC. and *E. rotundifolia* Casar. X ± SD (N).

	E. uniflora	*E. neonitida*	*E. punicifolia*	*E. rotundifolia*
Grains on open flowers	38.0^{A} ± 10.8(15)	32.4^{B} ± 30.0(23)	23.8C ± 11.9(13)	16.7^{D} ± 12.3(20)
Grains on bagged flowers	$11.4^{A'}$ ± 12.6(27)	$18.3^{B'}$ ± 12.4(23)	$24.5^{C'}$ ± 27.0(20)	$6.0^{D'}$ ± 19.5(20)

Comparison between the mean number of grains on open flowers and on bagged flowers: A-A'- SD ($t_{obs}=26.84 > t_{teor}=2.06$); B-B'- NSD ($t_{obs}=2.04 < t_{teor}=2.06$); C-C'- NSD ($t_{obs}=0.08 < t_{teor}=2.09$); D-D'- SD ($t_{obs}=2.72 > t_{teor}=2.09$). SD - significantly different, NSD - not significantly different.

Table 3. Main reproductive characters in *Eugenia uniflora* L., *E. neonitida* Sobral, *E. punicifolia* (Kunth) DC. and *E. rotundifolia* Casar. X ± SD (N).

	E. uniflora	*E. neonitida*	*E. punicifolia*	*E. rotundifolia*
Ovules/ovary	20.8 ± 3.5(20)	33.8 ± 5.6(20)	28.5 ± 6.2(20)	20.8 ± 2.9(20)
Seeds/fruit	1.6 ± 1.1(20)	1.5 ± 0.7(20)	1 ± 0.0(20)	1.08 ± 0.28(25)
Seeds/ovule	7.6%	4.4%	3.5%	5.19%
Fruits/flower	14.96%(127)	17.17%(64)	16.4%(61)	3.6%(56)
Predation ratio	72.22%(50)	98.54%(205)	98.4%(537)	97.14%(108)
Fecundity rate	0.0114	0.0075	0.0057	0.0019
Total reproductive success	0.32%	0.011%	0.009%	0.0052%

2000). Several authors, among them Levin (1972), Bawa & Webb (1984), Wiens *et al.* (1987) and Dalling & Hubbell (2002), argue that the pollination mechanism can be a limiting factor for fruit and seed abortion and consequently, in the reproductive success of a species. However, the low S/O ratio in *E. uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* is not directly linked to inefficient pollination, because the hand-crossing tests did not increase seed production per fruit.

Seed abortion in the species studied may be caused by genetic factors, since the low S/O ratio is a characteristic tendency in *Eugenia* (Wyk & Lowrey 1988; Beardsell *et al.* 1993a, b; Proença & Gibbs 1994; Lughadha & Proença 1996). Many species have a low S/O ratio, mainly due to hereditary characteristics acquired during the evolutionary process and, consequently, a limited amount of seeds per fruit can be an advantage for their dispersal (Casper & Wiens 1981; Charlesworth 1989; Dalling & Hubbell 2002).

In *E. uniflora* and in *E. neonitida*, species that have fruits with more than one seed (Tab. 4), we evaluated the relationship between the number of seeds and fruit mass. In *E. uniflora*, 74.63% of the fruits analyzed (N=67) had just one seed and 25.37% contained two or more. In *E. neonitida*, 81.03% of the fruits had one seed and 18.97% had two (N=58). In short, in *E. neonitida* and *E. uniflora*, an increasing number of seeds corresponds to an increase in fruit mass and in total seed mass per fruit; however, the individual mass of each seed decreases only slightly. This indicates that there is a larger energy investment in fruits with more than one seed. However, the proportion of fruits with these characteristics is smaller than that of fruits with just one seed (Tab. 4). These results indicate a selective pressure in favor of fruits with just one seed, although the number of seeds is a limiting factor in the creation of a seed bank and consequent reproductive success (Dalling & Hubbell 2002). These authors, as well as Cardoso & Lomônaco (2003), state that seed mass is positively related to seedling survival.

Fenner (1985) reports that plants inhabiting stable environments tend to invest more in seeds with qualities that favor their establishment and seedling survival, than in dispersal. *E. uniflora*, *E. neonitida*, *E. rotundifolia* and *E. punicifolia* display this tendency because their fruits have few seeds and a larger mass (Tab. 4, 5).

Ratio fruit/flower – Fruit production under natural conditions in *Eugenia uniflora*, *E. punicifolia* and *E. neonitida* was very similar, around 15%, different from that observed in *E. rotundifolia*, 3.6% (Tab. 1). The hand cross-pollination treatment yielded a higher

Table 4. Fruit and seed mass (gr) of *Eugenia uniflora* L., *E. neonitida* Sobral, *E. punicifolia* (Kunth) DC. and *E. rotundifolia* Casar. X ± SD (N).

	E. uniflora	*E. neonitida*	*E. punicifolia*	*E. rotundifolia*
Fruits/general	2.95 ± 1.03(67)	7.31 ± 3.66(58)	0.73 ± 0.15(25)	5.78 ± 1.05(15)
1-seeded fruit	2.69 ± 0.86^{A}(50)	6.96 ± 3.62^{D}(47)		
2-seeded fruit	3.69 ± $1{,}16^{A'}$(17)	8.79 ± $3.60^{D'}$(11)		
Unitary seeds - general	0.49 ± 0.22^{B}(87)	1.21 ± 0.52^{E}(58)	0.21 ± 0.06(25)	2.0 ± 00.4(15)
Seed from 1-seeded fruit	0.53 ± $0.20^{B',C}$(50)	1.26 ± $0.57^{E',F}$(47)		
Seeds from 2-seeded fruit	0.93 ± $0.46^{C'}$(17)	2.04 ± $0.67^{F'}$(11)		
Each seed from 2-seeded fruits	0.43 ± 0.23(37)	1.08 ± 0.35(22)		

Comparison among the mean mass: A-A'- SD ($t_{obs}=3.7>t_{teor}=2.01$), B-B'- NSD ($t_{obs}=0.32<t_{teor}=2.01$), C-C'- SD ($t_{obs}=5.0>t_{teor}=2.01$), D-D'- NSD ($t_{obs}=1.5<t_{teor}=2.01$), E-E'- NSD ($t_{obs}=0.92<t_{teor}=2.01$), F-F'- SD ($t_{obs}=4.2>t_{teor}=2.01$). SD - significantly different, NSD - not significantly different.

Table 5. Insect fruit and seed predators in *Eugenia uniflora* L., *E. neonitida* Sobral, *E. punicifolia* (Kunth) DC. and *E. rotundifolia* Casar. (ni - not identified).

	Coleoptera	Diptera	Hymenoptera	Heteroptera
E. uniflora	Larva (ni)	*Drosophyla* sp. Tephritidae	Eurytidae *Atta sexdens rubropilora*	_
E. neonitida	Larva (ni) Nitidulidae Chrysomelidae	*Drosophyla* sp.	_	Pyrrhocoridae
E. punicifolia	Larva (ni)	*Drosophyla* sp.	_	_
E. rotundifolia	Larva (ni)	*Drosophyla* sp.	_	_

fruit-set value than that of natural pollination for all the species studied, although only *E. uniflora* had significantly different values (Tab. 1).

Bawa & Webb (1984), Burd (1994) and Cunningham (2000), among others, suggest that the differences between natural and hand cross-pollination fruit/flower ratios indicate pollinator efficiency. However, Dogterom *et al.* (2000) observed that even the transfer of a great amount of pollen grains to the stigma may not maximize fruit production, since other factors could be associated with this process, such as limited maternal energy resources (Charlesworth 1989).

Our data are in agreement with Wiens *et al.* (1987) and Charlesworth (1989), who report that most of the species tend to produce many more flowers than the number of fruits that they can sustain. The natural fruit-set depends on the species and on the natural condition in the area. According to Wyk & Lowrey (1988), fruit production in the African species of *Eugenia* varies from 22 to 66% and in *E. dysenterica*, a species from central Brazil, it was 6.8% (Proença & Gibbs 1994).

The energetic factor is one of the main reasons for the limited fruit-set in the four species studied here, since in most of the stigma pollen grains analyzed, we found a sufficient amount to fertilize most of the ovules (Tab. 2). The low fruit-set in these species can be considered a result of a selective pressure in favor of the most vigorous fruits, and an adjustment in the nutrient supply to sustain fruit and seed development (Bawa 1974).

Fecundity rate – The four species studied here had a very low fecundity rate (Tab. 3). According to Wiens *et al.* (1987), outbreeding species have a lower fecundity rate than that of inbreeding species, due to the selection that occurs among genetically different embryos from cross pollination, in which the less vigorous ones do not grow. This was not observed in the four species studied here, because *Eugenia neonitida*, although self-incompatible, had a higher fecundity rate than that found in *E. punicifolia*, a self-compatible species (Tab. 3). The extremely low fecundity rate of the *Eugenia* species is linked mainly to the characteristically low S/O ratios reported in this genus (Wyk & Lowrey 1988; Proença & Gibbs 1994; Lughada & Proença 1996).

Seed and fruit predation – *E. uniflora*, *E. punicifolia*, *E. neonitida* and *E. rotundifolia* had a high fruit and seed predation ratio (Tab. 5), mainly due to Coleoptera larval development inside the seeds and fruits. *E. uniflora* is also parasitized by Tephritidae (Diptera) and Eurytidae (Hymenoptera) larvae, common in Myrtaceae species (Lima 1916; Lughadha & Proença 1996; Menezes *et al.* 2001).

Although galls have not been quantified in this study, their occurrence was observed in the flowers and fruit of *E. uniflora*. Maia (2001) described these galls as belonging to the Cecidomyiinae subfamily (Diptera) and reported they are not common in *restinga* fruits, occurring in only 3.9% of the species analyzed.

E. uniflora seeds are also predated by ant cutters (*Atta sexdens rubropilora* Forel, 1908). On one occasion, a group of five ants was observed carrying a single seed, partially predated, inside their nest. According to Levey & Byrne (1993), ants are antagonistic and mutual, since they consume some seeds and disperse others. *E. neonitida* is also parasitized by young individuals belonging to the Pyrrhocoridae family (Heteroptera) (Tab. 5). Schuh & Slater (1995) report that many species of this family are specialized in fruit and seed predation. The only Coleoptera larvae that emerged from the conditioned seeds were those found in *E. neonitida* fruits. These insects belong to the Chrysomelidae and Nitidulidae families and act mainly as seed and fruit predators of several species (Buzzi 2002; Bronstein *et al.* 2003).

Drosophyla sp. larvae parasite the fruit of the four species studied; however, they neither affect seed viability, since they do not consume, nor contribute to dispersal. In a large part of the predated seeds of *Eugenia uniflora*, *E. neonitida*, *E. punicifolia* and *E. rotundifolia* found in the litter, only the testa remained, with their contents totally or partially consumed. Coleopetra, Diptera and Hymenoptera larvae grow mainly inside the seeds of the four species, consuming most of their contents, thus affecting their germination viability.

Fruit and seed predation in these species is a decisive factor affecting their reproductive success, given that the predation ratio in litter seeds is very high. *E. uniflora* had the lowest rate, with 72.22% of predated seeds, and *E. neonitida* the highest, with 98.54% (Tab. 3). This difference may be associated with the germination behavior of these species, since, according to Zamith & Scarano (2004), *E. uniflora* germinates rapidly, whereas *E. neonitida*, *E. punicifolia* and *E. rotundifolia* are slow-germinating seeds.

The longer duration of the seeds in the substratum increases the likelihood of predators and parasites attacking them (Yanes & Segovia 1993; Silva *et al.* 2003), thus the recalcitrant seeds of these four species do not contribute to seed banks. Many species of Myrtaceae have recalcitrant seeds (Maluf *et al.* 2003; Cardoso & Lomônaco 2003). The loss of a large portion of the seeds, due to predation, is common in several habitats and is one of the main factors limiting the reproductive success of a species. In many cases, seed availability is drastically reduced, affecting population recruitment (Mack 1998; Wenny 2000; Cunnigham 2000; Silva *et al.* 2002; Mahoro 2003; Cardoso & Lomônaco 2003).

Total reproductive success – The total reproductive success in the four species studied is shown in Tab. 6. The total index was very low (Tab. 3), varying from 0.0052% in *E. rotundifolia* to 0.32% in *E. uniflora*. Our estimates on how many flowers are needed to produce a single seed yielded surprising values: *E. uniflora* needs a mean of 312.5 flowers, *E. neonitida* 9090.9, *E. punicifolia* 11111.1 and *E. rotundifolia* 19230.8.

Mass flowering phenology ensures that low total reproductive success may not be a serious problem for the population dynamics of these species, because, depending on the individual and on population size, thousands of flowers are produced at each flowering (Silva & Pinheiro 2007). However, in all of the stages of the reproductive process of a species, selective forces can act in favor of the most resistant individuals, promoting the natural selection process (Darwin 1859).

Table 6. Summary of the reproductive success stages of *Eugenia uniflora* L, *Eugenia neonitida* Sobral, *Eugenia punicifolia* (Kunth) DC., and *Eugenia rotundifolia* Casar. Fr/Fl: ratio fruit/flower found in controls experiments (table 1), S/Fr: number of seeds per fruit (table 3), S/O: number of viable seeds at the end of the reproductive process in relation to the total number of ovules in 100 hypothetical flowers.

	E. uniflora	*E. neonitida*	*E. punicifolia*	*E. rotundifolia*
Number of initial flowers	100	100	100	100
Number of fruits obtained (Fr/Fl)	16.40	14.96	17.17	3.6
Number of seeds obtained (S/Fr)	164.0	23.9	25.5	3.88
Predation ratio	98.4%	72,2%	98.5%	97.1%
Number of viable seeds	0.26	6.65	0.37	0.11
Number of initial ovules (ovules in 100 flowers)	2850.0	2075.0	3380.0	2080.0
Total reproductive success (S/O)	0.009%	0.32%	0.011%	0.0052%

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