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Florivory and sex ratio in *Annona dioica* St. Hil. (Annonaceae) in the Pantanal at Nhecolândia, southwestern Brazil

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RESUMO – (Florivoria e Razão Sexual em *Annona dioica* St. Hil. (Annonaceae) no Pantanal da Nhecolândia, Brazil). *Annona dioica* é uma espécie arbustiva de até dois metros de altura, amplamente distribuída nos cerrados. Esta espécie é conhecida por oferecer muitas flores, mas produzir poucos frutos. Enquadra-se como androdióica, possuindo indivíduos machos e hermafroditas na população. O objetivo deste estudo foi registrar a razão sexual e comparar a taxa de florivoria entre flores masculinas e hermafroditas. A coleta de dados ocorreu em campos de pastagem da Fazenda Nhumirim, consistindo na observação de arbustos floridos. Foram registrados 147 indivíduos masculinos e 71 hermafroditas, os quais apresentavam um total de 194 e 94 flores durante o período de estudo, respectivamente. A razão sexual observada foi de 2,07 indivíduos masculinos para cada hermafrodita, bem como 2,06 flores masculinas para cada flor hermafrodita. A taxa de florivoria foi significativamente maior em flores hermafroditas que em masculinas, com 33,0% (n = 31) e 25,7% (n = 50), respectivamente (G = 14,83; 1gl; p < 0,001). A média do peso fresco de 50 flores de ambos os sexos foi de 8,38 ± 2,40 g ($\bar{x}$ ± EP; n = 50) para flores masculinas e de 6,93 ± 2,68 g ($\bar{x}$ ± EP; n = 50) para hermafroditas, sendo significativamente diferentes (t = 2,479; df = 49; p = 0.017). Portanto, o baixo número de frutos encontrados nesta espécie pode ser explicado pela razão sexual, maior herbivoria em flores hermafroditas e provavelmente, pela ausência de polinizadores.

Palavras-chave: *Annona dioica*, Annonaceae, Curculionidae, florivoria, razão sexual

ABSTRACT – (Florivory and sex ratio in *Annona dioica* St. Hil. (Annonaceae) in the Pantanal at Nhecolândia, southwestern Brazil). *Annona dioica* St. Hil. is a species that grows to approximately 2 m tall and is very widespread in the cerrados. Individual plants of this androdioecious species produce numerous hermaphroditic or male flowers, but few fruits. The aim of this study was to determine the sex ratio among the plants and to compare the frequency of herbivory between male and hermaphroditic flowers. The fieldwork was done by studying flowering plants in grasslands used as pasture for cattle at Fazenda Nhumirim. One hundred and forty-seven male plants and 71 hermaphroditic plants were examined and produced a total of 194 and 94 flowers, respectively, during the study period. The male:hermaphrodite sex ratio was 2.07:1, and was similar to the male:hermaphrodite flower ratio of 2.06:1. The frequency of florivory rate in hermaphrodites was significantly higher than in male flowers (33.0%, n = 31, and 25.7%, n = 50, respectively; G = 14.83; d.f. = 1; p < 0.001). The mean fresh weights of male and hermaphroditic flowers were significantly different (8.38 ± 2.40 g vs. 6.93 ± 2.68 g, respectively; $\bar{x}$ ± SEM; n = 50 each; t = 2.479; d.f. = 49; p = 0.017). These results indicate that the low fruit set in this species can be explained by the sex ratio, the greater herbivory of hermaphroditic flowers and the probable absence of pollinators.

Key words: *Annona dioica*, Annonaceae, Curculionidae, florivory, sex ratio

Introduction

The Annonaceae (Magnoliales), the largest of the primitive families of flowering plants (Cronquist 1981), has a pan-tropical distribution and contains 122 genera and approximately 2300 species (Barroso 1992). The pollination system of this family is typically cantharophilous (Gottsberger 1970; 1974; 1990; Norman *et al.* 1992), with flowers that are highly specialized for pollination by beetles. The flowers of these species are frequently large, with an elevated number of stamens and carpels, and show flattened and dense aggregations of the reproductive structures in which the petals often close over the flower center to form a floral chamber (Gottsberger 1974; 1990). In addition, the flowers have massive, thick, nutritious petals (Gottsberger 1988; 1990; A.C. Webber, data not published) and use thermogenic respiration (Gottsberger 1989b; 1990; 1994; A.C. Webber, data not published) to guarantee an elevated temperature

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inside the flower (Gottsberger 1986; 1989a; 1990; H.F. Paulino Neto, data not published) that may be 15 ºC above the surrounding air temperature (Gottsberger 1990; 1994). The heat generated by the flowers enhances the volatilization of characteristic odours that serve to attract beetles (Gottsberger 1986; 1989a; 1990; 1994; A.C. Webber, data not published). Although cantharophily is the predominant pollination system among annonaceous species (Gottsberger 1977; 1986; 1988; 1989a; b; 1990; 1994; Webber 1981; 1996; Falcão *et al.* 1982; H.F. Paulino Neto, data not published), other systems, such as pollination by thrips (Gottsberger 1994; H.F. Paulino Neto, data not published), flies (Gottsberger 1988; Norman *et al.* 1992), bees (Carvalho & Webber 2000) and cockroaches (Nagamitsu & Inoue 1997), also occur.

In Brazil, the Annonaceae are represented by 29 genera and about 260 species, including *Annona dioica* St. Hil., a deciduous shrub up to two meters high and with a wide distribution in the cerrado (Barroso 1992). *Annona dioica* is an androdioecious species in which there are individuals with hermaphroditic flowers and others with only male flowers (Richards 1986). In the Pantanal region, this species is produces many flowers but few fruits (Pott & Pott 1994), a phenomenon also observed in other annonaceous species in the wild (Free 1993).

There has been increasing interest in assessing the reproductive output of plants in the last two decades (Stephenson 1981; Sutherland 1986; Ehrlen 1991; Herrera 2000), particularly because many flowering plants produce considerably more flowers than fruits (Stephenson 1981). The fruit set can be limited primarily by the resources available for investing in fruit production and secondarily by the availability of pollen (Stephenson 1981; Schemske and Horvitz 1988; Herrera 1991). In this context, variation in the frequency of flower visitation by pollinators will have little effect on the fruit set but can be important in determining the number of fruits produced by some plants (Schemske & Horvitz 1988; Kwak & Jennersten 1991; Cunningham 1995; Herrera 2000). Flower visitors have unequal effects on fruit production (Dieringer 1992; Cunningham 1995; Herrera 2000; Paulino Neto unplublished data). While some transfer pollen, others may decrease the quantity of pollen transferred or may damage flowers (Schemske and Horvitz 1988; Herrera 2000). The destruction of flowers by floral herbivores is an important cause of a low fruit set (Inouye 1982; Schemske and Horvitz 1988; Olesen 1992; Cunningham 1995).

The purpose of this work was to determine the male:hermaphrodite sex ratio in *A. dioica* and to quantify the rate of herbivory in the flowers of this species.

Materials and methods

Floral herbivory was studied in September 2001 at Fazenda Nhumirim, a 4.300 ha experimental station belonging to the Centro de Pesquisas Agropecuárias do Pantanal (Embrapa) and located 150 km from the city of Corumbá, in the Nhecolândia Pantanal, in the state of Mato Grosso do Sul, Brazil (18°59'S; 56°59'W). The region consists of "cordilheiras" and "capões" of semideciduous forests or cerrados separated by grasslands. The soils of this region consist of sand, silt or sandy clay derived from fluvial or lacustrine sedimentation deposited per periodic or occasional inundations.

These data were collected in two grasslands used as pasture for cattle. All flowering shrubs of *A. dioica* were observed, with the number of individuals and the number of flowers of each sex being recorded. The sex of the plants was determined by direct observation of the flowers and the frequencies of the two sexes were compared using the G-test (Zar 1996). The flowers were classified as untouched or damaged based on the extent of herbivory (florivory), with floral damage being recorded when there were petal perforations or the reproductive organs were injured. The frequency of floral damage in the reproductive organs of male and hermaphroditic flowers was compared using the G-test (Zar 1996). Fifty male flowers and 50 hermaphroditic flowers were collected and weighed to determine whether there was any difference in the mean dry weight of the two floral types; the values were compared using a *t* test (Zar 1996).

Sporadic observations of flower visitors were made from October 5 to 13, 2001 at Fazenda Nhumirim and Fazenda Rio Negro (19º34'S; 56º14'W), a property belonging to Conservation International in the Nhecolândia Pantanal. Four hours of daily observations resulted in a total of 36 h of observations. However, since two observers working separately were involved, the overall number of hours was 72. The floral visitors were collected and identified.

Results

Annona dioica is an androdioecious plant in which some individuals have hermaphroditic flowers while others have only male flowers that are 2-3 cm in diameter, with greenish to yellowish petals. From the total of 218 individuals recorded in the present study, 147 were male and 71 were hermaphroditic, which corresponds to a male:hermaphrodite ratio of 2.07:1. The 147 males produced 194 flowers, while the 71 hermaphrodites produced 94 flowers during the study period (total of 288 flowers), which corresponded to a ratio of 2.06:1 that was very similar to the sex ratio of 2.07:1. The mean number of flowers per individual (1.32) was the same in both sexes (Tab. 1).

Table 1. Abundance and florivory of hermaphrodite and male flowers of *A. dioica* St. Hil. at Fazenda Nhumirim, Nhecolândia, MS.

	Male	Hermaphrodite	Total
Total number of individuals	147	71	218
Total number of flowers	194	94	288
Number of damaged flowers	50 (25.7%)	31 (33.0%)	81
Damage restricted to petals	29 (14.9%)	18 (19.2%)	47
Damage to reproductive organs	21 (10.8%)	13 (13.8%)	34

From the total of 288 flowers, 81 were damaged by a beetle species and this injury was higher than expected in hermaphroditic flowers (Fig. 1). Among the 81 injured male and hermaphroditic flowers (male: n = 50; hermaphroditic: n = 31), damage to the petals (58%; n = 47) was greater than in the reproductive organs (42%; n = 34; Tab. 1). However, the total florivory rate (damage to floral traits) in hermaphrodites was significantly higher than in male flowers, i.e. 33% (n = 31) vs. 25.7% (n = 50), respectively ($G = 14.83$; d.f. = 1; $p < 0.001$; Tab. 1). In addition, the damage to hermaphroditic flowers was higher than expected while in male flowers it was lower than expected (Fig. 2). In contrast, the number of flowers with damage to their reproductive parts (androecium and/or gynoecium) was significantly higher than expected in hermaphrodites (13.8%, n = 13) and lower than expected in male flowers (10.8%, n = 21) (Tab. 1; $G = 11.22$; d.f. = 1; $p < 0.001$). The mean dry weights of the male and hermaphroditic flowers were 8.38 ± 2.40 g and 6.93 ± 2.68 g ($\bar{x}$ ± SEM; n = 50 each), respectively, and differed significantly ($t = 2.479$; d.f. = 49; $p = 0.017$).

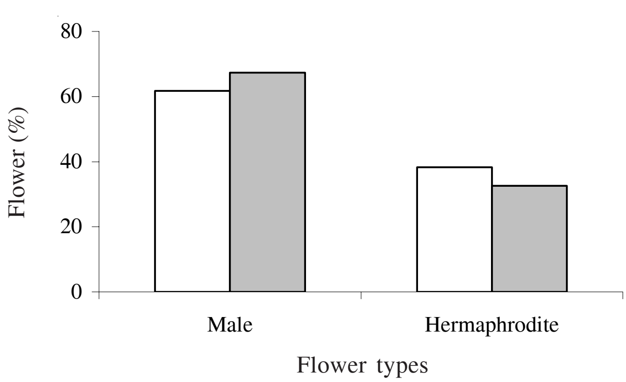

Figure 1. The percentages of available and damaged flowers of *A. dioica* St. Hil. at Fazenda Nhumirim, Nhecolândia, MS. □ = Damaged (n = 81); ■ = Available (n = 288).

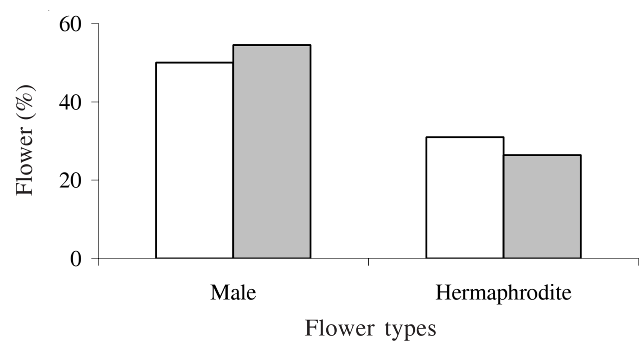

Figure 2. The expected and observed percentages of damaged flowers of *A. dioica* at Fazenda Nhumirim, Nhecolândia, MS. □ = Observed (n = 81); ■ = Expected.

At both study sites, only beetles of the genus *Conotrachelus* (Curculionidae) were found visiting flowers of *A. dioica*. These weevils were observed gnawing petals and feeding on androecia or gynoecia. The females deposited their eggs on the reproductive parts of male and hermaphroditic flowers. In addition, many flower buds showed damage by *Conotrachelus* larvae, which were often found inside the floral chamber and in many immature fruits.

Discussion

In the Pantanal region, *Annona dioica* produces many flowers, but few individuals produce fruit (Pott & Pott 1994). The male:hermaphrodite sex and flower ratios is approximately 2:1, and this can explain the low fruit set in this species. Although many *A. dioica* individuals flower extensively, only a third of these plants are hermaphroditic (have female organs) and can produce fruit. In addition, the greater herbivory of hermaphroditic flowers may also contribute to the small crop (in this study, 42% of the flowers had a damaged gynoecium, which makes fruit development inviable).

Male *A. dioica* flowers are heavier than hermaphroditic flowers. This finding agrees with

Gottsberger (1989a), who also found male flowers to be larger and heavier. Since beetles commonly visit only male flowers (Rodrigues *et al.* 1993), the expectation was that male flowers would be more attacked since they provide a greater food resource. However, hermaphroditic flowers were attacked more frequently, perhaps because, in addition to the androecium seen in male flowers, they also have a gynoecium and offer their visitors a stigmatic exsudate (protonectar). Damage to the reproductive organs, especially the gynoecium, can directly affect fruit production and may contribute to the low productivity.

The curculionid beetle *Conotrachelus* sp. was the only visitor to *A. dioica* flowers at both sites studied and was considerated a flower predator. According to Gottsberger (1988; 1989a), these beetles are frequent flower predators in the Annonaceae. This is an interesting finding since these plants are typically pollinated by beetles of the genus *Cyclocephala* (Gottsberger 1986; 1988; 1989a). The absence of *Cyclocephala* spp. on the studied plants may be a result of direct habitat destruction by humans. According to Bond (1994) and Steffan-Dewenter *et al.* (2002), the diversity and abundance of species decrease proportionally with the fragmentation and destruction of their natural habitats. In the present case, the use of natural grasslands as pasture may hinder the access of *Cyclocephala* to flowers or eliminate them from this region of the Pantanal. Another possibility is that *Cyclocephala* beetles occur only in preserved areas of the Pantanal and would therefore not be present in the two areas of pasture areas studied here.

In conclusion, the results of this study suggest that floral herbivory and a lack of effective pollinators of *A. dioica* flowers may reduce the fruit set in this species, which already has a naturally low productivity because of the skewed sex ratio. Habitat fragmentation and destruction could also negatively influence this insect-plant interaction. Future studies should examine the influence of habitat modification on the *Cyclocephala* population and on the pollination and fruit set of *A. dioica.*

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