# DISPERSÃO, FENOLOGIA E CONSERVAÇÃO DE
## *Annona crassiflora* Mart. (Annonaceae)

## DANIEL LEÃO BANDEIRA DE MELO

## 2009

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**DISPERSÃO, FENOLOGIA E CONSERVAÇÃO DE**
*Annona crassiflora* **Mart. (Annonaceae)**

> Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Curso de Doutorado em Engenharia Florestal, área de concentração em Ciências Florestais, para a obtenção do título de "Doutor".

Orientadora

Profa. Dra. Dulcinéia de Carvalho

**LAVRAS**
**MINAS GERAIS – BRASIL**
**2009**

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|---|---|
| Profa. Dra. Carla Rodrigues Ribas | UFLA |
| Prof. Dr. Edvaldo Aparecido Amaral da Silva | UFLA |
| Profa. Dra. Flávia de Freitas Coelho | UFLA |
| Prof. Dr. Flávio Nunes Ramos | UNIFAL |


Profa. Dra. Dulcinéia de Carvalho
UFLA
(Orientadora)

**LAVRAS**
**MINAS GERAIS – BRASIL**

À Gaia, que no infinito de possibilidades nos gerou,

**DEDICO.**

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# SUMÁRIO

# RESUMO

MELO, Daniel Leão Bandeira de. **Dispersão, Fenologia e Conservação de** *Annona crassiflora* **Mart. (Annonaceae)**. 2009. 65 p. Tese (Doutorado em Ciências Florestais) – Universidade Federal de Lavras, Lavras, MG. [*]


*Annona crassiflora* Mart. (*Annonaceae* conhecida por marolo ou araticum) é um importante componente do estrato arbóreo do cerrado e pertence a um gênero importante ecologicamente e economicamente. Os dados de dispersão e fenologia do marolo foram coletados em uma propriedade rural no município de Carmo da Cachoeira, Sul de Minas gerais. O local de estudo é representativo do bioma cerrado, sendo modificado a um sistema silvopastoril com marolo e *Brachiaria decumbens*. Neste trabalho, foram estudados 50 frutos de marolo, naturalmente dispersos na área (barocoria), para identificar seu dispersor de sementes; realizamos o monitoramento da fenologia reprodutiva e vegetativa, incluindo testes de polinização. Besouros "rola bosta", relacionados à *Dichotomius aff. ascanius* (Harold), foram observados enterrando sementes de marolo juntamente com a polpa do fruto. A fenologia revelou um comportamento bimodal no florescimento da espécie (duas floradas por ano), embora tenha sido observado apenas um evento de dispersão de frutos por ano. Esse comportamento foi associado à herança filogenética e à interação com o polinizador *Cyclocephala atricapilla*. O teste de polinização sugeriu uma baixa taxa de polinização cruzada na população analisada, provavelmente devida a baixa incidência do polinizador. A partir desse resultado, propôs-se para a área de estudo, a reintrodução de espécies coexistentes de *Annona (A. coriacea, A. dioica, A. monticola)* que compartilham o mesmo polinizador, a redução da intensidade de pastoreio e a implantação de áreas de cerrado próximo.


Palavras chaves: *Annona crassiflora, dispersor de sementes, fenologia, polinização.*

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[*] Orientador: Profa. Dra. Dulcinéia de Carvalho – UFLA.

# ABSTRACT

MELO, Daniel Leão Bandeira de. **Dispersion, Phenology and Conservation of *Annona crassiflora* Mart. (Annonaceae)**. 2009. 65 p. Thesis (Doctor Program in Forest Science) – Federal University of Lavras, Lavras, MG.*

*Annona crassiflora* Mart. Annonaceae, locally called araticum or marolo) is an important component of Cerrado canopy and belongs to a genus ecologically important and commercially valuable. Data collection was carried out in a private ranch in Carmo da Cachoeira, South of Minas Gerais. The study area is cerrado, but nowadays is a silvopastoral system with marolo and *Brachiaria decumbens*. In this work, we analyzed 50 fruits of marolo, after natural dispersion (barochory), aiming to identify the seed disperser; we also realized studies of reproductive and vegetative phenology, including pollination tests. Seeds were buried from the fallen fruit, together with the pulp, by dung beetle species related to *Dichotomius aff. Ascanius*. The species show a bimodal flowering behavior during the year. This phenomenon could be explained by the center of origin of the Annonaceae and the selective force toward to maintain the population of pollinator and to guarantee the pollination function. The pollination tests showed that fruit set can be improved by hand cross-pollination. Other possible way to improve the fruit set is to enhance natural pollination by reducing the intensity of animal grazing, preserving natural forests and forest fragments in the vicinity of the agroforestry system, and reintroducing *A. coriacea, A. dioica, A. monticola*, co-occurring species also pollinated by the dynastine scarab beetles, *Cyclocephala atricapilla*.

Key-words: *Annona crassiflora*, seed disperser, phenology, pollination

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# CAPITULO 1

## 1 INTRODUÇÃO GERAL

O bioma Cerrado é único, com uma alta riqueza de espécies e de fisionomias. Estima-se que a flora da região possua 10 mil espécies, algumas ainda pouco estudadas, de alto potencial alimentar, madeireiro, combustível, agroindustrial, forrageiro, medicinal e ornamental (Avidos & Ferreira, 2008). Segundo Gottsberger & Silberbauer-Gottsberger (2007), a classificação do cerrado como savana neotropical negligencia a sua complexidade, sendo que esta definição só se aplica a parte do cerrado e não se adequa aos extremos, como o cerradão e o campo sujo. O cerrado possui uma complexidade de espécies de flora e fauna e de fisionomia muito superior a qualquer savana do mundo. Essa complexidade única em nosso planeta já foi em grande parte perdida, restando cerca de 20% da área original (Mittermeier & Russel, 2000). Embora os dados de perda de cobertura original do cerrado sejam controversos, nota-se que o bioma continua em processo de destruição e fragmentação.

Na busca de um melhor entendimento das consequências desse processo de destruição e fragmentação de habitats, a equipe do Laboratório de Conservação Genética de Espécies Arbóreas do Departamento de Ciências Florestais da UFLA vem elaborando e implantando projetos envolvendo estudos interdisciplinares para orientar estratégias de conservação e/ou recuperação de espécies do bioma cerrado. Nesse sentido, foi elaborado um projeto multidisciplinar para a conservação de *Annona crassiflora*. A tese aqui apresentada é a primeira parte desse projeto.

*A. crassiflora*, popularmente conhecida como marolo, araticum, cabeça-de-negro, cascudo e pinha-do-cerrado, pertence à família Annonaceae e foi escolhida como planta alvo desse projeto por ser uma das espécies arbóreas do

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cerrado com maior potencial alimentar e econômico. Os frutos são grandes, apresentando cerca de 20 cm de diâmetro, pesando até 3 kg, sendo muito apreciados pelas pessoas. Na época da dispersão dos frutos, de fevereiro a abril, estes são comercializados em feiras livres e beiras de estradas. Dos frutos pode-se retirar a polpa e utilizá-la na produção de geléias, sorvetes, doces e licores. Além disto, *A. crassiflora* possui princípios ativos com potencial de uso na indústria farmacêutica para a produção de medicamentos utilizados para o tratamento de câncer. Apesar de sua importância, a espécie vem sofrendo com a ocupação e conseqüente fragmentação do cerrado.

A presente tese sintetiza o conhecimento gerado para a espécie *A. crassiflora* e está estruturada em três artigos, conforme a opção de formatação de dissertações e teses do Programa de Pós-Graduação em Engenharia Florestal. O primeiro artigo trata da dispersão da espécie e será submetido à *Seed Science Research*. O segundo artigo trata da fenologia da espécie e pretendemos submetê-lo à *Canadian Journal of Botany*. O terceiro artigo trata da conservação da espécie, propondo uma alternativa de manejo silvopastoril, e foi preparado para envio ao *Journal of Applied Ecology*.

Os resultados e discussões aqui obtidos servirão de base para discutir os dados de biologia molecular que estão sendo gerados e que em breve também serão publicados. Como o projeto tem o enfoque conservacionista e visa realizações práticas, um banco de germoplasma ativo *in situ* foi implantado na área de estudo, Carmo da Cachoeira.

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# CHAPTER 2 - DUNG BEETLES AS SEED DISPERSERS OF *Annona crassiflora* MART. (ANNONACEAE)

# 1 ABSTRACT

To determine if dung beetles are disperser of *Annona crassiflora*, 50 dung beetles 'nests' were investigated from February 2008 to January 2009 in Carmo da Cachoeira, Southeast Brazil. Depth, germination and viability of buried seeds by dung beetles were evaluated. Seeds from the fallen fruit were buried together with the pulp by dung beetle species related to *Dichotomius aff. ascanius* and eventually by *Phanaeus kirbyi*. However, no seedling was found and every evaluated seed was dead, indicating that seed predation can be more significant for *A. crassiflora* recruitment than seed dispersion. This is the first description of dung beetles dispersing seeds directly from fallen fruits.

Key-words: *Annona crassiflora, Dichotomius, seed dispersion, seed predation, Cerrado.*

# 2 INTRODUCTION

Plant recruitment seems to be an interaction between seed dispersion in suitable site and seed predation (Orrock et al., 2006; Schupp, 2007). Harper (1977) defines as 'safe site' the local area, determined by the size of the seed, that provides necessary stimuli for breaking seed dormancy, required conditions for germination, such as oxygen and water, and the absence of hazards such as predators and pre-emergence pathogens.

*Annona crassiflora* (*Annonaceae*, locally called *araticum* or *marolo*) is a characteristic tree of the Cerrado formation (Lorenzi, 1998), which resembles other savannas such as those in Africa (Rather et al., 1997). The species produces enormous (up to 3 kg) fleshy fruits, with many seeds, that are appreciated for its strong smell and flavour. The species has seeds with morphophysiological dormancy that can remain in the soil seed bank for more than 20 months (Silva et al., 2007). However, the seed cannot endure severe water shortage, being necessary to be incorporated into soil seed bank in a viable time to avoid seed desiccation (Rizzini, 1973). The natural seed disperser of *A. crassiflora* has not been identified yet. Thus, the main objective of this paper is to identify the seed disperser of *A. crassiflora*.

# 3 MATERIALS AND METHODS

## 3.1 Study Site

The study was conducted in an agroecosystem at a private ranch located in Carmo da Cachoeira, south of Minas Gerais State, Brazil (21º26'S; 45º05'W). The study site was originally cerrado *sensu stricto*, but today it is a grass field wooded with *A. crassiflora* trees randomly spread, which is used for grazing and collecting *A. crassiflora* fruits. The presence of young plants of *A. crassiflora* was observed, indicating the natural regeneration of the species in the area.

## 3.2 Plant Species

The *A. crassiflora* fruits are dispersed from February to March, in the end of wet season (Rizzini, 1973). The *A. crassiflora* seed is about 2 cm long, coated hardly (Rizzini, 1973), and has morphophysiological dormancy (Silva et al., 2007). The seeds remain dormant in the soil seed bank during the whole dry season (from March to August) and only germinate in the beginning of the wet season in September (Rizzini, 1973; Silva et al., 2007).

## 3.3 Seed Fate

To confirm if dung beetles is *A. crassiflora* seeds disperser, after barochory dispersion of the fruit, 50 fruits, already dispersed on the ground, were monitored from February 15th, 2008 to January 10th, 2009, and the observations were made every week. Germination and seed viability in the dung beetle's 'nests' were also verified in January, when 7 dung beetle's 'nests' were dug, the seeds were counted its depth was measured up to 20 cm deep. Seed

viability was made in the field, once viable seeds have clear endosperm and good smell and dead ones have dark endosperm and smell bad. To make it easier to understand, in this paper the term 'nest' will be used to describe the tridimensional ground space under the fruit used by dung beetles to bury pulp and seeds of *A. crassiflora*

# 4 RESULTS

The first group of animals to be attracted by the ripped fruits fallen on the ground was the dung beetle species related to *Dichotomius aff. ascanius* (Harold). These *D. aff. ascanius* drill holes in the tough leathery pericarp to remove and bury pulp and seeds. Up to six specimens were found in each of the 50 marked fruits, and in all cases the specimens were females (Figure 1). Occasionally*, Phanaeus kirbyi* Vigors were found digging pulp and seeds and, in this species, both males and females were found. We observed high intensity of seed predation, especially by weevil beetles *Curculionidae*.

The dung beetles buried pulp and seeds immediately below the fruit. We found a mean of 39 seeds per marked fruit, buried by dung beetle in each 'nest', at different depths. Most of the seeds (67%) were found at depths of 1-5 cm, 23% at depths of 6-10 and 10 % at depths of 11-20. Seed depth distribution is summarized in the Table 1 below.

TABLE 1    Mean deep distribution of *Annona crassiflora* seeds found in *Dichotomius ascanius* 'nests' in Carmo da Cachoeira, South of Minas Gerais - Brazil.

| Seed depth | < 5 cm | 6-10 cm | 11-20 cm |
|---|---|---|---|
| Seeds per 'nest' | 26 | 9 | 4 |
| % of buried seeds | 67% | 23% | 10% |

No seedling was observed in any 50 dung beetle's 'nests', and every seed found in the 7 dung beetles 'nests' were predated.

FIGURE 1   Dispersed marolo, *Annona crassiflora* fruit, being consumed by female dung beetle related to *Dichotomius aff. ascanius.*



FIGURE 2  *Annona crassiflora* seed buried by dung beetles at 3 cm deep.

# 5 DISCUSSION

In this paper, for the first time, it's report that dung beetles disperse seeds right from fallen fruits on the ground, by burying seeds together with the pulp under the fallen fruit. However, no seedling was found from buried seeds, but it's confirmed that the seeds were buried right from the fallen fruit.

*A. crassiflora* seed has morphophysiological dormancy and the seeds remain dormant in the soil seed bank during the dry season in the Cerrado biome, from March to August (Silva et al., 2007). Rizzini (1973) observed that even the seeds that have deeper dormancy cannot endure severe water shortage, indicating that the seeds must be buried right after releasing the fruit and kept buried to avoid desiccation until completing germination. Rizzini (1973) observed that seeds of *A. crassiflora* taken from naturally desiccated fruits, or even seeds which were subjected to drying conditions in nature for only a few days did not germinate. On the other hand, seeds that were not expose to drying conditions, and buried in wooden flats with sand, had germination between 75-100 % (Rizzini, 1973). Silva et al. (2007) had a similar result with *A. crassiflora* seeds buried at 5 cm depth, three days after seed had released the fruit, without been submitted to severe dry condition. These authors also observe that seeds stay dormant in the soil, at 5 cm depth, for two seasons, germinating 60% on the first year and 12% on the second year. These results suggest that seeds of *A. crassiflora* have long life span, been able to remain in the soil seed bank longer than a year, once it is buried right after released from the fruit, as soon as possible, without been submitted to severe dry condition.

The dung beetles were the first visitors to the fallen fruits on the ground. They remove the tough leathery pericarp to access the pulp and start to remove and bury it. Occasionally, some seeds are buried with the pulp and these seeds are protected from desiccation condition, as it's described by Rizzini (1973) to a

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successful germination. Although, no germination was found, many seeds were buried in a compatible time under necessary condition by dung beetles.

This study takes over one season germination of *A. crassiflora* that goes from October to January after seed dispersion. Silva et al. (2007) demonstrated that these seeds can remain dormant in the seed soil bank for at least two seasons, being possible to find seed germination in the next season.

Seed predation was very intense in the field since dispersion. This high predation could explain no germination in the field. The observation that all the seeds found in the 'nests' were predate indicate that seed predation in the field condition can reach 100%. Orrock et al. (2006) testing the importance of dispersal and predator limitation in affecting the distribution of pokeweed, *Phytolacca americana* in South Carolina - USA, demonstrated that dispersal limitation was negligible across a large scale and that seed predation can be an important determinant of landscape-level plant abundance at large-scale.

Dung beetles are known in the literature as secondary seed dispersers, usually using mammals' defecation as resource, and promoting a significant reduction in seed predation and seedling competition (Feer, 1999; Andersen, 2001; Nichols et al., 2008). Although the use of ripen fruits as food for adults and larvae of dung beetles be known to occur (Nichols et al., 2008), dung beetles dispersing seeds from fallen fruits has not been reported yet.

It was concluded that *A. crassiflora* seeds are buried by dung beetle species related to *Dichotomius ascanius* and occasionally by *Phanaeus kirbyi*, right from the fallen fruit. The germination result in the marked 'nests' did not allow us to answer if dung beetles are positive or negative disperser of *A. crassiflora.* However, the mean amount of seeds buried at suitable depth for germination and the 100% of seed mortality, most by predation, found in the 'nests' in January, give us the idea that seed predation can be more significant for plant recruitment than seed dispersion for *A. crassiflora.* It would be relevant

to conduct additional experiments under natural vegetation conditions for longer period of time, and to test whether differences in seed burial depth are biologically important. This is the first description of dung beetles as primary seed disperser.

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# CHAPTER 3 - PHENOLOGY OF *ANNONA CRASSIFLORA* MART. (ANNONACEAE)

# 1 ABSTRACT

Phenological behaviour among tropical trees has been a long-standing question for ecologists. Plant phenology in cerrado trees seems not to be by a single cue and appears to be driven by biotic and abiotic interaction over a phylogenetic heritage. To interrelate the whole complexity involved in the phenological behaviour of tropical trees, it seems to be necessary to deeply study a single species. The main objective of this study is to describe the phenology of adult trees of *Annona crassiflora*, an important component of cerrado canopy that belongs to a genus that is both ecologically important and commercially valuable. We recorded environmental cues, intensity and synchrony of phenological events of leafing, flowering and fruiting, and interrelated them using Spearman's correlation. *Annona crassiflora* is a brevideciduous species. Rain fall is the main resource to support vegetative growth and day length plays an important role in vegetative bud break, working as a trigger for leaf flush. The species shows a bimodal flowering behaviour during the year and anthesis synchrony only shows correlation with photoperiodic variation, coinciding with the proximity to the equinoxes. This phenomenon could be explained by the center of origin of the *Annonaceae* and the selective force toward to maintain the population of pollinator and guarantee the pollination service. *A. crassiflora* disperses its fruit during the wet season. It appears that in *A. crassiflora* reproduction is designed to suit the most favourable conditions for fruiting and seed dispersion rather than for flowering.

Key words: **Annona *crassiflora*** Mart., *phenology, equinox, climate seasonality, biotic interaction.*

# 2 INTRODUCTION

Phenological behaviour among tropical trees has been a long-standing question for ecologists (Borchert, 1983; Wright & Cornejo, 1990; Williams et al., 1997; Borchert, 2005; Zimmerman et al., 2007). Lieth (1974) defined phenology as the study of (i) the rhythm of repetitive biological events, (ii) the biotic and abiotic causes of these events, and (iii) the relationship between phenophases. The distribution over time of life-history events, such as leaf flush and fall, flowering, fruiting, and seed dispersal, can greatly affect individual and population fitness or species survival (Augspurger, 1981). Phenological patterns can influence the reproductive successes of a species (Okullo et al., 2004) and studies about this could be important to the conservation of tree genetic resources and forestry management as well as for a better understanding of plant species.

Interpretation of plant phenology has suggest that synchrony in tropical trees is caused by a strong interaction between abiotic and biotic factors (Augspurger, 1981; Mduma et al., 2007). In Serengeti National Park, for savannas trees, rainfall is the primary determinant of reproduction synchrony, but the activities of predators and dispersers can increase the degree of synchrony (Mduma et al., 2007). Rainfall seasonality also can shape phenological patterns (Reich & Borchert, 1984; Wright & Schaik, 1994). However, it is established that biological interactions (Hamann, 2004) and phylogenetic relations have influence on phenology too.

Water is a key resource in cerrado region, characterized by strong rainfall seasonality. Available water is required to ensure the positive turgor pressures necessary to expand growing cells during leaf flush, flowering and fruit development (Zimmerman et al., 2007). The cerrado soils are generally deep and well drained (Franco, 2002). Superficial soil layers dry out during the

dry season that extends from May to September, while deeper soil layers exhibit a much higher degree of water constancy throughout the year (Franco, 2002). It is assumed that most woody species of Cerrado vegetation have deep roots that access constantly available water and that deep roots are a pre-requisite for maintaining high leaf water potentials ($\Psi_l$) during the dry season, when most trees produce a new crop of leaves (Walter, 1971; Sarmiento, 1984).

Seasonal variation in the availability of water and light should place physiological and thus evolutionary constraints on the phenologies of tropical forest plants (Schaik et al., 1993; Wright, 1996). Day length is a function of solar declination (Borchert et al., 2005; Renner, 2007; Yeang, 2007), being the only environmental signal that is constant every year. Because of this, photoperiodicity is considered to control the phenological patterns of many tree species in tropical rain forests (Morellato et al., 2000; Borchert et al., 2005). The seasonal rhythm of plant phenology is thought to be driven, in large part, by changes in the sun´s zenith (Windsor, 1990).

Moved by the complexity showed above, phenology in cerrado trees is not driven by a single cue; it seems to be a fitness response to biotic and abiotic factors over a phylogenetic heritage. To interrelate the whole complexity involved in the phenological behaviour of tropical trees, it seems to be necessary to deeply study a single species.

*Annona crassiflora* (Annonaceae) is an important component of cerrado canopy and belongs to a genus that is both ecologically important and commercially valuable. Its fruit has economical value (Ribeiro et al., 2000) and compounds purified from its seeds showed, *in vitro*, cytotoxicity to human lung carcinoma and melanoma cells (Santos et al., 1996) and biological activity against *Aedes aegypti* larvae (Lima et al., 2006), whereas extracts obtained from its leaves showed antifungal properties (Silva et al., 2001). Despite to its importance, the species is under threat due to the modern agriculture and fire.

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The main objective of this study is to describe the phenology of adult trees of *A. crassiflora*.

# 3 MATERIALS AND METHODS

## 3.1 Study Area

The present work was carried out in the municipality of Carmo da Cachoeira, south of Minas Gerais State, Brazil (21º26'S; 45º05'W), 980 m above sea level. The soil of the study site is a distrophic oxisol on a flat topography. This area suffered many changes over the last 30 years and the natural vegetation, a cerrado *sensu stricto*, was changed to a silvopastoral system with pasture of *Brachiaria decumbens* wooded with adults trees of *A. crassiflora* left on the field. The area is used for grazing and to collect fruits of *A. crassiflora.*

The climatic classification by Köppen method for the region is Cwa; subtropical mesothermic with wet "summer" from October to March, and dry "winter" from April to September. Dantas et al. (2007), using Thornthwaite method, using the climate data from 1990-2004, classified the climate as **B$_2$rB'$_3$a'**, which means humid climate, with little water deficit, mesothermic, with annual evapotranspiration of 956 mm. Dantas et al. (2007) collected climate data from the same meteorological station than we did.

## 3.2 Study Species

The *A. crassiflora* occurs in the whole cerrado or "cerradão" at 400-1200 m above sea level, belonging to the superior stratum, usually 6 m to 8 m tall. The trees are often tortuous, bark thick, corky, fissured; the leaves are ovate to obovate, glabrous and glaucous. The flowers are yellowish hermaphroditic and protogynous, with one night rhythm flowering (Gottsberger, 1989). The species is mainly or exclusively pollinated by the dynastine scarab beetles,

*Cyclocephala atricapilla* (Gottsberger, 1989). To confirm the beetle species in Carmo da Cachoeira, we collected some beetles found in the flowers and sent to a Dynastidae specialist.

**3.3 Data Collection**

**3.3.1 Environmental cues**

Monthly values for precipitation, maximum and minimum temperatures, day length, insulation and air humidity were obtained from the station number 83687 of the Brazilian national meteorological service, located about 25 Km from the study area. All weather readings were taken at 9:15 h and 21:00 h Brazilian time.

**3.3.2 Recording of phenological events**

Leafing, flowering, and fruiting phenologies (phenophases) from 23 adults trees were recorded from September 2006 to December 2008. Monitoring was done every month from April to August, and every week from September to March, for leafing and flowering phenophases for better characterization. Intensity and synchrony of each phenophase was scored. Intensity is described by the semi-quantitative Fournier index (Fournier, 1974) from 0 to 4 ($1 \leq 25\%$, $2 = 26\text{-}50\%$, $3 = 51\text{-}75\%$, $4 \geq 76\%$ cover of the tree) and to determine the synchrony we used the percentage of presence and absence of each phenophase.

## 3.4 Data Analysis

We used the Spearman's correlation coefficients to express the relationship between phenological events (phenophases) and climatic variables assessed (Zar, 1999). For each phenophase, Spearman's correlation coefficients were also used to show the synchrony between consecutive years during monitored seasons (2007/2008 and 2008/2009). We consider significant $P$-level < 0.001.

# 4 RESULTS

## 4.1 Environmental Cues

During the study time the average annual rainfall was 1451.4 mm (1293.2 in the rainy season and 158.2 in the dry season); relative air humidity (70.6 ± x 9.2%); day mean air temperature (20.4 ± 2.9 ℃) and average solar irradiance increases by 40–50% over the dry season months (April to September). The Figure 1 illustrates the environmental cues data used in the Spearman's correlation.

Day length was the environmental cue that had more significant Spearman's correlation ($P < 0.001$) with the phenophases (Tables 1 and 2). Phenophases synchrony and intensity had a similar Spearman's correlation, excepted for leaf covering intensity that seems to be more correlated with environmental cues than leaf covering synchrony (Tables 1 and 2).

## 4.2 Leafing

The species has a unique leaf flushing event per year, which begins when the plant is leafless, in September and ends around 120 days later, in January, when the leaves are completed expanded. This period corresponds to the transition of dry to wet season. Leaf flush synchronism and intensity show a similar result to Spearman's correlation ($P < 0.001$) with rain fall, solar irradiance and day length (tables 1 and 2). Leaf flush between consecutive observed years had a strong Spearman's correlation (r = 0.943, $P$-level = 0.000004), suggesting that this phenophasis seems to be well modulated by the invariable environmental cue (ie. day length). Leaf flush had no significant Spearman's correlation ($P < 0.001$) with leaf loss.

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The first visual signal of leaf senescence was in January and the first leaf abscission was in March; the plants gradually loses their covering from March to August when abscission intensifies leading to completed deciduous in September (figure 2). These months correspond to the dry season, once the wet season is normally from October to March. Leaf covering intensity had more significant Spearman's correlation ($P < 0.001$) with environmental cues than leaf covering synchrony (tables 1 and 2).

The species shows a briefly deciduous behaviour, losing all the leaves during a short period of time (< 3 weeks) every year. The sampled individuals remain leafless for 14 days in the mean, but some individuals remain leafless for only 7 days and one remains this way for 25 days. Leaf loss was the most correlated phenophase with the environmental cues tested; leaf loss synchronism and intensity show a similar result to Spearman's correlation ($P < 0.001$) (tables 1 and 2). Day length seems also to play an important role in leaf loss, being significantly correlated ($P < 0.001$) with simultaneous data, one month late data and two months late data (tables 1 and 2).

Irradiance had an unexpected result, being negatively correlated with leaf flush and positively correlated with leaf loss (tables 1 and 2). It means that the species flushes new leaves when the irradiance begins to reduce and starts to lose senescence leaves when the irradiance begins to increase.

## 4.3 Flowering Activity

A characteristic feature of the species is to keep preformed flower buds dormant during the dry season. The bud dormancy overlap with the proximity to equinox and bud development until anthesis lasted for 6 to 7 weeks.

During monitoring periods, two flowering events were observed every year, characterizing a bimodal flowering activity (Figure 2). The main event,

which generates fruits until dispersion, lasted for 12 weeks – from the second week of September to the first week of December – during the change from dry to wet season (Figure 2). The secondary event is less intense, lasting for 8 weeks between April and May; in the beginning of dry season with early abortion of pods. These two events coincide with the proximity with the equinox, what is confirmed by the significant Spearman's correlation ($P < 0.001$) of anthesis synchrony exclusively with day length (tables 1). Anthesis intensity was also Spearman's correlated ($P < 0.001$) with solar irradiance (Table 2). The beetle identification confirmed the *Cyclocephala atricapilla* Mannerheim (1819), and even not monitoring the pollinator, the presence of them in both flowering events was observed.

Anthesis between observed years had Spearman's correlation ($r = 0.873$, $P$-level $= 0.0002$), suggesting that this phenophasis is most modulated by the invariable environmental cue (ie. day length). Anthesis had no significant Spearman's correlation ($P < 0.001$) with leaf loss.

## 4.4 Fruiting Episode

The fruit development lasted for around 140 days when the young green fruit with 0.6 cm of diameter became a big yellowish fruit with until 16 cm of diameter. The first green fruit appeared in October and fruit dispersion started in the second half of February and lasted until the beginning of May (figure 2), in the end of the rainy season.

Fruit dispersion had Spearman's correlation with rain fall of two and three month later (tables 1 and 2), suggesting that fruit development is water dependent, which explains the early abortion of pods after the secondary flowering event.

# 5 DISCUSSION

## 5.1 Vegetative Behavior

*Annona Crassiflora* is a brevideciduous species, losing gradually its leaves during the dry season, reaching the complete leaf less during a short period of time in the drier moment of the year, that corresponds to the end of the dry season. Recent studies suggest that most species in cerrado are isohydric (Franco, 1998; Meinzer et al., 1999; Bucci et al., 2005), maintaining nearly constant minimum $\Psi_l$ throughout the year, despite changes in soil water availability and atmospheric condition (Tardieu & Simmoneau, 1998). The leaf loss behaviour observed in *A. crassiflora* is likely to be part of the mechanism that helps to maintaining nearly constant minimum $\Psi_l$ throughout the year.

According to Sarmiento & Monasterio (1983), most of the woody cerrado species produce new leaves and flowers during the dry period, indicating available water in the rhizosphere. Deciduous species usually have deeper root systems than species with persistent foliage in cerrado (Jackson et al., 1999), which would make deciduous species less dependent on rainfall than evergreen species for expanding new leaves. Deep rooting is especially important in seasonally dry environments, such as cerrado, because it enables plants to access water stored deeply in the soil during periods of low water availability, and allows them to maintain transpiration and carbon fixation (Sarmiento et al., 1985; Meinzer et al., 1999).

Even if there isn't empirical data of depth of *A. crassiflora* root, it seems it has deep root system, as expected for deciduous cerrado species (Sarmiento, 1984; Castro & Kauffman, 1998; Jackson et al., 1999). The species leaf flush during the dry season probably relied on subsoil water reserves, which buffer trees against prolonged climatic drought. The establishment of new foliage in the

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beginning of rain season is likely to optimize photosynthetic gain in the relatively short wet growing season.

According to Reich & Borchert (1984), at the end of the dry season, deciduous species of Costa Rica tropical forest lose all their leaves so that transpiration is reduced and reserves of water in the soil are sufficient to allow plant re-hydration. In Australian savannas, the reduction of the leaf transpiration area of deciduous species would improve plant water status and allow plants to flower or produce new leaves during the dry season. Thus, the elimination of water deficit within the woody plant initiates the leaf flush (Eamus & Prior, 2001). In *A. crassiflora*, the peak of leaf loss in September, reaching complete defoliation during two weeks, may improve plant re-hydration and allow leaf flush.

The species has a unique leaf flushing event per year, in the late dry season. It is in agreement with other deciduous cerrado species that seem to have a reduced shoot growth pattern in time during the wet season (Damasco et al., 2005). This behaviour can allow plants to be full of stored carbon in roots and shoots during the dry season as in temperate deciduous species in winter (Larcher & Thomaser-Thin, 1988). These reserves accumulated during the favourable period could be mobilized in the beginning of the wet season, being used to leaf flush (Damasco et al., 2005).

Leaf flush had a strong Spearman's correlation between observed years, what suggests that this phenophasis is also modulated by the invariable environmental cue (ie. day length). Leaf fall seems to play an important role in plant re-hydration, what allows leaf flush, but seems not to be the signal trigger that induces leaf flush, that had no significant Spearman's correlation ($P < 0.001$) with leaf loss. Other information that indicates the importance of day length in the vegetative behaviour is the fact that it was the environmental cue that most correlated with leaf loss (tables 1 and 2).

Radiation, precipitation and solar declination are closely linked in the tropics. The sun heats most effective when at its zenith, but heat continues increasing after the sun passes its zenith. This heat builds up a loose of low pressure and cloudiness, the Intertropical Convergence Zone, where vapour rises to condense as rain in convective storms. The risen air, cold and deprived of moisture, diverges to either side of this zone, sinks, and forms drying trade winds bringing air to replace that which has risen (Lauer, 1989). This way, the results suggest that, even if rain fall is the main resource to support vegetative phenology, it seems not to be the main trigger to induce changes in *A. crassiflora* canopy.

Graham et al. (2003) recently showed experimentally that, in Panama, wet-season cloud cover limits annual $CO_2$ assimilation of *Luehea seemannii* Tr. & Planch. (Tiliaceae), a late successional canopy tree species. This confirms that tropical trees are light-limited during cloudy periods. Thus, everything else being equal, tropical trees should be under selection to flush new leaves during periods of high irradiation (Graham et al., 2003). However, *A. crassiflora* had an opposite behaviour, producing new leaves when the irradiation begins to reduce and starts to lose old leaves when the irradiation begins to increase. A hypothesis to explain this behaviour is the multi-factorial selective forces acting together, which reduces the importance of irradiation in relation of all, and that wet-season cloud cover in cerrado region is not dense as in Panama and does not represent a severe constraint to plant $CO_2$ assimilation.

**5.2 Reproductive Event**

**5.2.1 Flowering**

*Annona crassiflora* shows a bimodal flowering behaviour during the year. The main flowering event goes from late September to early December, what corresponds to the beginning of wet season. However, the secondary flowering event occurs in the beginning of dry season, what shows no correlation with rain fall, but with photoperiodic variation, coinciding with the proximity to the equinoxes.

Borchert et al. (2005) argue that, if it were solely photoperiodic control that induces flowering, the timing at the species level should be identical in time and space and should occur mainly around the equinoxes. Yeang (2007) argues that, if a physiological episode in a perennial plant occurs consistently at the same time every year, it is likely to be linked to some recurrent meteorological event. In *A. crassiflora,* the arguments for photoperiodic control are the observed bimodal shape of flowering peaks around the equinoxes and the significant Spearman's correlation ($P < 0.001$) between observed years. Thus, there is evidence that photoperiodic is the "flowering synchronizer" of *A. crassiflora*.

Renner (2007) and Yeang (2007), both using rubber tree (*Hevea brasiliensis*) as a study model, argue that sunshine triggers flowering synchrony in the tropics. Yeang (2007) argues that there is a reasonable likelihood that synchronous flowering in the rubber tree (and other tropical trees sharing similar flowering characteristics) is connected with some aspect of solar radiation arising from the movement of the earth around the sun. According to Renner (2007), there is evidence that annual changes in insulation provide the external cue that synchronizes flower bud break in *H. brasiliensis*. First, rubber trees start flowering at the same time every year, making it unlikely that temperature,

rainfall, cloud cover, or periods of drought could be the triggers as they do not occur with sufficient regularity. Secondly, data from rubber plantations in the northern and southern subtropics of South America, Africa and Asia show that flowering coincides with high insulation and not temperature or rainfall. The third line of evidence is the fit between the bimodal peak in incoming solar radiation and the bimodal flowering in *H. Brasiliensis* in some places. However, most of *H. brasiliensis* populations flower only once a year, around the spring equinox (Yeang, 2007).

At the equator, incoming solar radiation (insolation) is due to radiation intensity, once day length is almost constant through the year (Yeang, 2007). At the equator, insolation peaks twice a year at the equinoxes, when the midday sun is directly overhead (Borchert et al., 2005). Yeang (2007) noted that rubber trees growing near the equator and in the subtropics flowered when solar radiation intensity was high. She inferred from this that the cycle of solar radiation intensity was responsible for synchronous anthesis and blooming in *H. brasiliensis.*

How trees perceive daily changes in insolation is not clear yet (Renner, 2007). At last, the interaction of light absorbed by the various photoreceptors with the expression of circadian clock-dependent transcription factors must determine flowering (Simpson et al., 1999; Yanovsky & Kay, 2003).

Walker (1971) combined an investigation of pollen and floral morphology and phytogeography to produce a natural classification of the *Annonaceae*. He observed strong evidence for the origin of the *Annonaceae* family in the American tropics (or possibly Africa), with the major center in the Amazon Basin and a secondary center in Central America (Walker, 1971). This origin from tropical region around the Equator could be a possible explanation for the origin of the bimodal flowering behaviour. This could be a phylogenetic

heritage from an ancestral that comes from a tropical region around the equator (i. e. Amazon Basin) that had a bimodal flowering behaviour.

Synchrony is also considered the result of coevolution of trees with pollinators and seed predators (Borchert, 1983). It has been argued that peaks of flowering occur during periods of high pollination activity, and that overlapping with different flowering peak periods in related species reduce competition for pollinators and assure a continuous source of nectar to pollinators (Janzen, 1967). This biotic hypothesis supposes that simultaneous flowering leads to increased pollination and reproductive success for some species (Mduma et al., 2007). Climate is the primary determinant but the activities of predators, pollinators and dispersers increase the synchrony (Mduma et al., 2007).

*A. crassiflora* has a Cantharophily pollination syndrome, being pollinated by *Cyclocephala atricapilla* (Gottsberger, 1989). This author got surprised to find so little specificity between this dynastid sacarab beetle species and different *Annona* species. He found that *C. atricapilla* was the main or exclusive pollinator of *A. coriacea, A. crassiflora, A. dioica,* and *A. monticula,* four co-occurring species that grew close together. Although all four species flowered in the rainy season from October to January and there was a broad overlapping of the flowering periods, they showed a staggered flowering with clearly separated flowering peaks (Gottsberger, 1989). The author argues that the staggered flowering of *Annona* spp. seems to be rather a development for diminishing competition between the different species. This phenomenon is described as shared-pollinator hypothesis (Wright & Calderon, 1995).

The flowering behaviour of the *A. crassiflora* leads us to an ecological question; why does the species remain with bimodal behaviour, flowering in the transition of the rain to the dry season, when the conditions are not favourable to fruit development and early abortion of pods occur? During the field work the presence of the pollinator was observed in both flowering events. This

phenomenon can be an explanation for conserving the bimodal behaviour. Flowering in unfavourable period of the year can be an important resource to the pollinator, what could be important to maintain the population of pollinator to the next flowering event.

After all, it can be hypothesized that flowering behaviour of *A. crassiflora* comes from its phylogenetic heritage, being synchronized by photoperiodic variation, (i.e. proximity to the equinox) that induces bud break and anthesis; shared-pollinator hypothesis increases synchrony; all supported by deep root system in deep water available soil associated with leaf fall behaviour that maintains nearly constant minimum $\Psi_l$ throughout the year. The selective force to maintain pollinator service is the main reason for the species keep flowering in unfavourable period of the years.

### 5.2.3 Fruiting

*A. crassiflora* has zoochorous diaspore syndrome (Chapter 1), dispersing its fruit during the wet season, from February to April. In recent studies at community level, Batalha & Martins (2004) show that most of zoochorous trees in the cerrado of Central Brazil fruited from September to December, during rainy season. For *A. crassiflora,* it is a good strategy to fruit during the wettest period of the year, when its fresh fruit can be kept attractive for longer time (Gottsberger & Silberbauer-Gottsberger, 1983; Batalha et al., 1997; Batalha & Mantovani, 2000; Batalha & Martins, 2004).

*A. crassiflora* has a large and tasty fruit (can weight more than 3 Kg) and during its development the trees has a high carbohydrate demands. This high demand during fruit development coincide with the most favourable period of the year, the wet season, and with new foliage covering, that is more photosynthetic efficient. The significant Spearman's correlations ($P < 0.001$) of

fruit dispersion with rain fall at two and three months later (tables 1 and 2) confirm these observations. The high fruit demand could explain the early abortion of pods observed after the secondary flowering, in the beginning of the dry season.

In Chapter 1, the dung beetle species related to *Dichotomius ascanius* (Harold) is describes as primary seed disperser of *A. crassiflora,* by burying the seeds under necessary conditions to seed germination. According to this author, these dung beetles bury the seeds right from the fruit in a short period of time, avoiding the dispersed seeds from drying conditions. In tropical forests with a pronounced seasonality in rainfall, adult dung beetles show lower abundance or may even disappear during the dry season and dispersal by dung beetles is likely non-existent during the dry season in such forests (Janzen, 1983).

Fruiting in *A. crassiflora* has correlation with rainfall what does not happen with flowering, and this may be because fruit dispersion occurs towards the end of the rains, whereas flowering begins in the end of dry season. Thus, it appears that *A. crassiflora* reproduction is designed to suit the resource conditions for fruiting and seed dispersion rather than for flowering.

In this paper we described part of the fitness behaviour of *A. crassiflora*, being necessary to continue researching to better understand the reproductive system, plant recruitment and phylogenetic heritage of the species, trying to better understand how selective forces lead to *A. crassiflora*.

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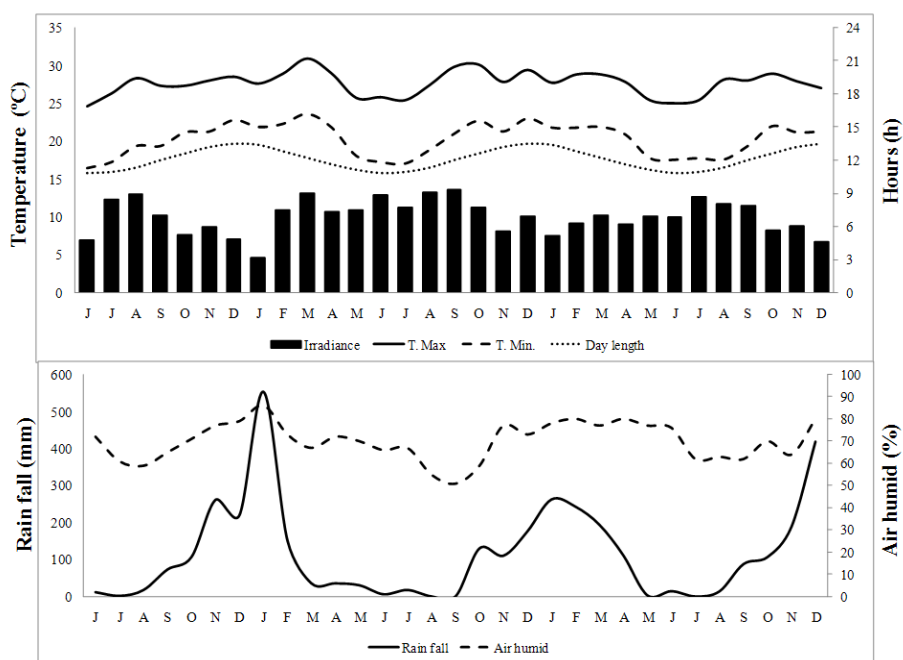
FIGURE 1  Environmental cues data – two years observation (June 2006 to December 2008) in Lavras – southeast Brazil.
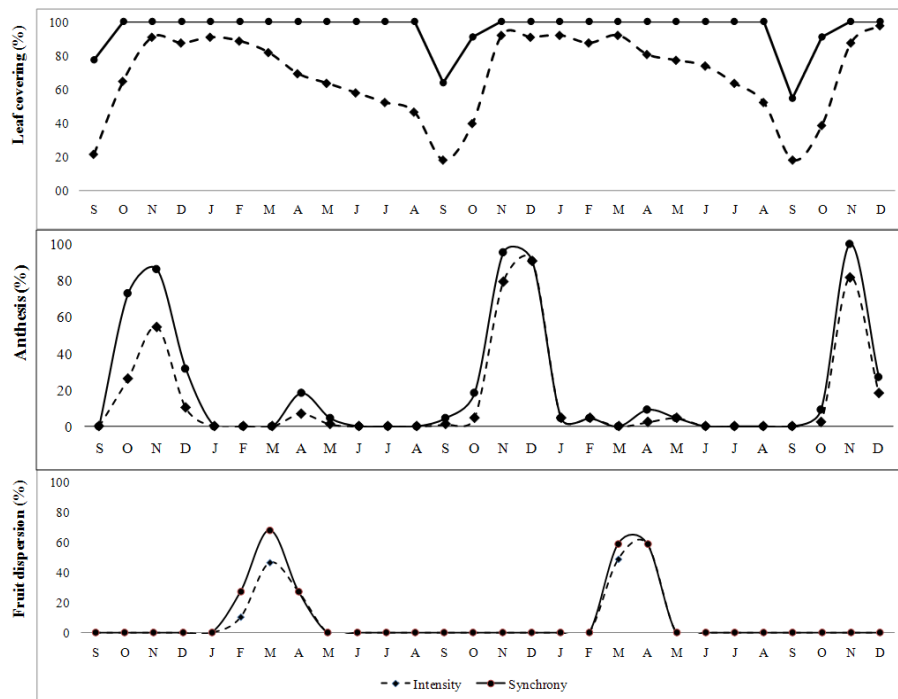
FIGURE 2 Intensity and synchrony of leaf covering, anthesis and fruit dispersion – two years observation (September 2006 to December 2008) in Carmo da Cachoeira – southeast Brazil.

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TABLE 1    Values of $r_s$ to significant Spearman correlations ($P < 0.001$) between phenophases synchrony and environmental cues in simultaneous data and preceding months (i.e. assuming one, two and three months lag).

| Phenophases | Simultaneous data | | | 1 month later | | | 2 months later | | | 3 month later | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Climate cues | $R_s$ | *P*-value | Climate cues | $R_s$ | *P*-value | Climate cues | $R_s$ | *P*-value | Climate cues | $R_s$ | *P*-value |
| Leaf loss | T. Min. | -0.780 | 0.00000 | T. Min. | -0.697 | 0.00004 | | | | | | |
| | Rain fall | -0.786 | 0.00000 | Rain fall | -0.822 | 0.00000 | | | | | | |
| | Air humid | -0.758 | 0.00000 | | | | | | | | | |
| | Irradiance | 0.782 | 0.00000 | Irradiance | 0.786 | 0.00000 | | | | | | |
| | Day length | -0.737 | 0.00001 | Day length | -0.835 | 0.00000 | Day length | -0.708 | 0.00003 | | | |
| Leaf flush | Rain fall | 0.657 | 0.00014 | | | | | | | | | |
| | Irradiance | -0.667 | 0.00010 | | | | | | | Air humid | -0.822 | 0.00000 |
| | Day length | 0.842 | 0.00000 | | | | | | | | | |
| Leaf covering | | | | Air humid | 0.610 | 0.00056 | T. Min | 0.600 | 0.00073 | T. Max | 0.627 | 0.00049 |
| | | | | | | | | | | Day length | 0.658 | 0.00098 |
| Anthesis | Day length | 0.590 | 0.00093 | | | | | | | | | |
| Fruit dispersion | | | | | | | | | | T. Min. | 0.597 | 0.00078 |
| | | | | | | | Rain fall | 0.615 | 0.00050 | Rain fall | 0.619 | 0.00039 |
| | | | | | | | | | | Day length | 0.663 | 0.00098 |

TABLE 2     Values of $r_s$ to significant Spearman correlations ($P < 0.001$) between phenophases intensity and environmental cues in simultaneous data and preceding months (i.e. assuming one. two and three months lag).

| Phenophases | Simultaneous data | | | 1 month later | | | 2 months later | | | 3 month later | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Climate cues | $R_s$ | $P$-value | Climate cues | $R_s$ | $P$-value | Climate cues | $R_s$ | $P$-value | Climate cues | $R_s$ | $P$-value |
| Leaf loss | T. min | -0.754 | 0.00000 | T. min | -0.696 | 0.00004 | | | | | | |
| | Rain fall | -0.789 | 0.00000 | Rain fall | -0.830 | 0.00000 | | | | | | |
| | Air humid | -0.786 | 0.00000 | | | | | | | | | |
| | Irradiance | 0.778 | 0.00000 | Irradiance | 0.786 | 0.00000 | | | | | | |
| | Day length | -0.705 | 0.00003 | Day length | -0.823 | 0.00000 | Day length | -0.732 | 0.00001 | | | |
| Leaf flush | Rain fall | 0.649 | 0.00018 | | | | | | | T. min | -0.589 | 0.000966 |
| | Irradiance | -0.646 | 0.00020 | | | | | | | Air humid | -0.805 | 0.000000 |
| | Day length | 0.837 | 0.00000 | | | | | | | | | |
| Leaf covering | T. min | 0.662 | 0.00012 | T. min | 0.690 | 0.00005 | T. min | 0.620 | 0.00043 | | | |
| | Rain fall | 0.718 | 0.00002 | Rain fall | 0.791 | 0.00000 | Rain fall | 0.605 | 0.00064 | | | |
| | Air humid | 0.791 | 0.00000 | | | | | | | | | |
| | Irradiance | -0.637 | 0.00027 | Irradiance | -0.746 | 0.00001 | | | | | | |
| | Day length | 0.631 | 0.00032 | Day length | 0.790 | 0.00000 | Day length | 0.771 | 0.00000 | | | |
| Anthesis | Irradiance | -0.610 | 0.00056 | | | | | | | Air humid | -0.596 | 0.00081 |
| | Day length | 0.628 | 0.00035 | | | | | | | | | |
| Fruit dispersion | | | | | | | Rain fall | 0.610 | 0.00057 | T. min | 0.593 | 0.00088 |
| | | | | | | | | | | Rain fall | 0.624 | 0.00039 |
| | | | | | | | | | | Day length | 0.661 | 0.00013 |

# CHAPTER 4 - LOW FRUIT SET IN A WOODEN PASTURE: A PROPOSAL FOR SILVOPASTORAL SYSTEM MANAGEMENT IN CERRADO REGION AT SOUTHEAST BRAZIL

# 1 ABSTRACT

Cerrado is a "hot spot" threatened by human occupation that changed its original vegetation into agriculture and exotic pasture, being today a fundamental production area in Brazil. The main challenge is to make the cerrado becomes economically viable so it can coexist better with human way of life. To better understand *Annona crassiflora* Mart. (Annonaceae)*,* Pollination, tests were done in in a wooden pasture. Hand cross-pollination had higher fruit set (17%) comparing with natural pollination (4%) and autogamous self-pollination (6%). This result suggests a lack in pollinator efficiency. The pollination tests showed that fruit set can be improved by hand cross-pollination. Other possible way to improve the fruit set is to enhance natural pollination by reducing the intensity of animal grazing, preserving natural forests and forest fragments in the vicinity of the agroforestry system, and reintroducing *A. coriacea, A. dioica, A. monticola,* co-occurring species also pollinated by the dynastine scarab beetles, *Cyclocephala atricapilla*.

Key-words: *Annona crassiflora*, breeding system, coleopteran, pollinator limitation, landscape ecology, conservation.

# 2 INTRODUCTION

In the last decades the growth of agriculture turned into an intensive occupation landscape especially with exotic crops and pasture. In Brazil, the cerrado biome is not an exception. The Cerrado is the most diverse savanna in the planet; it contains high numbers of local and regional endemic species (Ratter et al., 1997), being considered one of the earth's "hot spot" for the biodiversity preservation (Mittermeyer et al., 1999).

In a capitalist world with the higherst population ever in the history, a way to preserve ecosystems, natural or semi-natural, is to make it worth more with natural species compared with exotic crops or pasture. Species with economical and ecological values are particularly important in this process, consorting native trees with pasture in agro-ecosystems.

*Annona crassiflora* is a native cerrado tree species, belonging to the Annonaceae (Gottsberger, 1989). The fruit has economical value, and in the study area fruit selling gives extra money around U$ 2.000 per year, being an important resource to the owners. This species is an important component of Cerrado canopy and belongs to a genus that is both ecologically important and commercially valuable, so the knowledge about the ecology of fruit set is an important tool to manage agro-ecosystem with *A. crassiflora.*

When complex native ecosystems like cerrado are deforested and transformed into agro-ecosystems, innumerous changes can happen in many ways. One of them is the reduction of vegetation complexity and microclimatic variation (Morris, 2000); another one is the loss of abundance and species richness of flowering plants (Corbet et al., 1991). This structure simplification and the reduction of complexity, in particular through the action of grazing animals (Morris, 2000), bring many consequences, including declining pollination and declining fruit set (Cunningham, 2000a).

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Increasing flowering plant diversity has been argued to increase visitor diversity (Heithaus, 1974). Particular attention has been paid to create `attractive' (i.e. floriferous) environments (Morris, 2000). Landscape management must be tempered by full consideration of ecological factors and conservation aims and that 'practical' and 'theoretical' conservation needs to interact more effectively (Morris, 2000).

To establish this link between cerrado deforestation, declining pollination and fruit set, evidence that fruit set is limited by reducing pollinator service is required. In a way to propose landscape management of agro-ecosystem, the aim of this paper is to determinate the breeding system and pollen viability of *A. crassiflora* in a wooden pastured land in a dairy farm. It was hypothesized that low fruit set in natural pollination, compared with hand pollination, can be the consequence of low pollinator activity, what is expected to happen in deforested environment.

# 3 MATERIALS AND METHODS

## 3.1 Study Site

The present work was carried out under pasture condition in the municipality of Carmo da Cachoeira, South of Minas Gerais State, Brazil (21°26'S; 45°05'W). The climatic classification by Köppen method for the region is Cwa; subtropical mesothermic, at 980 m above sea level and annual average temperature of 20,4 °C, with wet "summer" from October to March, and dry "winter" from April to September, with annual average rain fall of 1460 mm (Dantas et al., 2007).

The study area was originally cerrado *senso strictu*, but today it is a grass field, of *Brachiaria decumbens* Stapf, with trees of *A. crassiflora* randomly spread, which is used for grazing and collecting the fruits of *A. crassiflora.*

## 3.2 Study Species

The *Annona crassiflora* flowers at the end of the dry season or in the beginning of the rainy season, normally when it does not have new leaves yet (Gottsberger, 1989). In Chapter 2, it was observed two flowering peaks during the year and this behavior was correlated with equinox proximity. *A. crassiflora* flowers are hermaphroditic and protogynous (Gottsberger, 1989). The species has a distinct one-night rhythm flowering. The flowers which open their petals during the day, in the morning or even in the early afternoon, usually reach their active female phase in the same evening and flowers which start opening their petals only in the late afternoon or evening, enter their active female phase only at dusk of the following night (Gottsberger, 1989).

## 3.3 Data Collection

The pollen viability was checked by collecting pollen of 10 flowers. They were stained with acetic carmine 1.2% (Radford et al., 1974). 5 glass slides for each flower were prepared and, 100 pollen gains of each glass slide were sampled, totalizing 500 pollen grains counted per flower under a microscope (Studar 40X). The results were expressed in percentage of viable pollens.

The pollination trials, designed to investigate the breeding system, were performed from October 2007 to March 2008 through the following treatments: a) manual cross-pollination - receptive female flowers which had been isolated were pollinated with pollen obtained from other individuals; afterwards they were bagged until the flowers withered and fruit development began; b) Autogamous self-pollination - isolated flowers within paper bags from the bud stage was left covered without external intervention until they reached the fruit-forming stage; c) Agamospermy - to observe the formation of fruits in the absence of male gametes, buds which had previously been emasculated were isolated until they reached the fruit-forming stage; d) Natural pollination - the number of fruits which developed under natural conditions of pollination was quantified. In all experiments, the flowers were marked from the flowering up to the fruit-forming stages, and for each test 50 flowers were used. The results were expressed in percentage of fruit set.

# 4 RESULTS

The counting of pollen grain stained shows high viability, with 83.76% of the grains viable. The pollination test showed a pollen dependence to fruit set, once agamospermy had no fruit set. The species is self-compatible and the test had 6% of fruit set by this manner. The natural pollination had a low fruit set (4%) if compared with manual cross-pollination (17%), which was the highest percentage of fruit set. The table 1 resumes the pollination test results.

TABLE 1     Results of pollination test with *Annona crassiflora* associated with pasture in a cattle farm in Minas Gerais, southeast Brazil

| Pollination test | Fruits/flower | % of fruit set | Green fruits | % of fruit abortion |
|---|---|---|---|---|
| Manual *cross-pollination* | 7/50 | 17 | 7 | 0 |
| *Autogamous self-pollination* | 3/50 | 6 | 11 | 73 |
| *Natural pollination* | 2/50 | 4 | 10 | 80 |
| *Agamospermy* | 0/50 | 0 | 0 | 0 |

**5 DISCUSSION**

The species has high percentage of viable pollens, around 83%, and the flowers produce a large amount of pollen. This result is similar to others cross-pollinated Annona, like *A. cherimola, A. muricata, A. squamosa* (George & Nissen, 1987).

The *A. crassiflora* pollen is a compound of four grains (Walker, 1971), known as tetrad pollen, which derived from the same meiotic divisions (Copenhaver, 2005). It represents a valuable resource because they allow a process known as tetrad analysis (Copenhaver, 2005). Tetrad analysis is a flexible tool that can be used to construct genetic maps (Copenhaver et al., 1998), to define centromere positions (Copenhaver et al., 1999), to detect chromosomal rearrangements (Ortiz & Vorsa, 2004), to measure crossover and chromatid interference (Copenhaver et al., 2002), and to reveal gametophytic mutations (Johnson & Mccormick, 2004).

The species does not produce fruit without pollination - agamospermy, but it is not self-incompatible, and produce 3 normal fruits by autogamous self-pollinated, one more than the control – natural pollination. The manual cross-pollination has the highest amount of fruit set, producing 7 normal fruits. These results suggest that the species is not self-incompatible, but produces a higher number of fruits by cross-pollination. These results are similar to *Annona squamosa* that is self-compatible but has higher fruit set by cross-pollination, indicating the possibility of late mechanism of self-incompatibility (Kiill & Costa, 2003). The high percentage of green fruit abortion in self-pollination test (73%) suggests that *A. crassiflora* has some late mechanism of self-incompatibility as suggested for *A. squamosa* by Kiill & Costa (2003). Other evidence of self-compatibility is the flowers of *A. crassiflora* that are intrafloral

protogynous (Gottsberger, 1989); what is associated with self-compatibility species (Bertin, 1993).

The difference between manual cross-pollination and the other tests suggest the low efficiency of the natural pollinator, once natural pollination had similar number to autogamous self-pollinated and lower than manual cross-pollination. The high percentage of green fruit abortion in natural cross pollination tests (80%), even higher than self-pollination tests (73%), indicates the low efficiency of pollinator service, once cross pollination test had zero percent of green fruit abortion. Probably, this can be consequence of cerrado deforestation that causes lack in *C. atricapilla* niche.

These Dynastid scarb beetles are the main or exclusive pollinators of many species from the genera *Annona* (Gottsberger, 1989). It seems that plant species that are pollinated by a narrow suite of visitors will be more vulnerable to the effects of habitat fragmentation on pollination and fruit production (Cunningham, 2000b). The pollinator crisis scenario warns that habitat destruction is reducing the abundance of pollinators, causing a decline in the rate plants that are pollinated in fragmented sites that will, in turn, lead to lower fruit production (Cunningham, 2000a).

Blanche et al. (2006) studying *Dimocarpus longan* crops, observed a positive relationship between initial longan fruit set and higher stingless bees visitation. This suggesting that enhanced pollination in longan near rainforest is because primarily from a more abundant supply of stingless bees from the rainforest. The seed set of animal-pollinated plants is determined by pollinator limitation and/or resource limitation. Low levels of fruit set under natural conditions suggest that pollination success in deceptive orchid is limited by the availability of pollinators (Matsui et al., 2001; Pellegrino et al., 2005).

Klein et al. (2003) observed that fruit set in lowland coffee (*Coffea canephora*) increased with the diversity and abundance of flower-visiting bees.

They observed that a bee community of 20 species or more led to a higher fruit set (95%) than a species-poor bee community of six species (70% fruit set). They stated that a species-rich and abundant bee assemblage will facilitate high pollination success in lowland coffee. This will increase fruit set and coffee yield. The authors recommended the farmers to encourage different species of bees through simple management measures such as growing coffee in shade beneath a variety of trees; by pruning trees to increasing levels of sunlight and numbers of flowering herbs; and by increasing the availability of nesting sites for solitary bees. Weed control and the use of herbicides should be kept to a minimum so that a diverse nectar and pollen resource is available to bees throughout the year. Natural forests and forest fragments should be preserved in the vicinity of coffee agroforestry systems (< 500 m) so that forest-nesting social bees can travel easily to the coffee fields to pollinate the flowers (Klein et al., 2003).

Arthropod assemblages are likely to be determined by a range of factors including resource availability, microclimate preferences and predator avoidance (Stork & Grimbacher, 2006). According to Gottsberger (1989), four co-occurring *Annona spp.* are mainly or exclusively pollinated by *Cyclocephala atricapilla.* The flowering period of these four co-occurring species, *A. crassiflora, A. coriacea, A. dioica, A. monticola,* goes from October to January, providing food and mating site for *C. atricapilla* during at least four months per year (Gottsberger, 1989).

In chapter 2, it was observed a bimodal flowering behavior in *A. crassiflora,* and a single fruit episode per year. It was hypothesized that a secondary flowering event, under inappropriate conditions for fruit development, could be linked with pollinator survival during an unfavorable period of the year. This secondary flowering occurs between April and May, and increases the

period of food and mating site for *C. atricapilla*, provided by the four co-occurring *Annona* species from October to January.

The study area had its natural vegetation changed for exotic pasture, being left adults trees of *A. crassiflora,* which provide flowers only in October and November (Chapter 2)*.* This reduction in flower resource for *C. atricapilla* during the year could contribute to the low efficiency of natural pollination test. Gazit et al. (1982), working with *A. cherimola,* found that at least four beetles per flower is required to obtain high-quality fruit set in Israel. When the number of insect pollinators is lower, it is necessary to manually pollinate flowers to produce commercially viable quantities of large well-formed fruit. Sjödin et al. (2008) found out in Sweden that low intensity or abandoned grasslands showed higher visitor richness than intensive grazing for beetles.

The results of several studies suggest that forest fragmentation affects the mating patterns and reproductive success of tropical tree species by reducing pollinator activity, pollen deposition, and outcrossing levels (Fuchs et al., 2003). Empirical evidence shows that forest fragmentation affects negatively the plant reproduction by reducing pollinator activity, pollen deposition, and fruit and seed set (Ghazoul et al., 1998; Cunningham, 2000a). Researchers have found a positive relationship between fruit set and forest-fragment size (Ghazoul et al., 1998; Cunningham, 2000b).

Land use intensification and habitat fragmentation can also contribute to pollinator reduction. Trampling by grazing animals directly kill plants and create areas with less plant cover (Morris, 2000). Because grazing vegetation becomes shorter, which entails lower plant structural complexity under intensive management, many invertebrates which are dependent on aerial structures may fail to survive (Morris, 2000). If grazing pressure is intense, it might be expected that flower abundance would become low and individual flowering plants scattered (Morris, 2000).

Duffey (1975b), studying the effects of treading and grazing on the composition, diversity and abundance of grassland invertebrate communities, emphasized the importance of management, or rather its cessation, to these animals. He also demonstrated that invertebrate animals are far more sensitive to treading than vegetation. Even treading of grassland by humans, as walkers, visitors or holiday makers, is also considered detrimental, at least when it is intensive. The major experimental study of the effects of human trampling on the invertebrates of grassland litter (Duffey, 1975a) has demonstrated the much greater sensitivity of invertebrate animals to such activity and even moderate trampling (5 treads/month) reduced coleopteran abundance by 82% after 12 months compared with untrampled controls. The presence of grazing animals modifies the vegetation at the soil surface by treading and the physical presence and movement of the grazing animal through the field layer have effects which have been little investigated (Morris, 2000).

To achieve long-term sustainability of agricultural landscapes, it is vitally important that their management includes commitment to conserving native biodiversity, and the ecosystem services it provides, as well as improving agricultural production (Blanche & Cunningham, 2005).

The pollination tests show that *A. crassiflora* fruit production can be improved by manual cross-pollination once natural pollination shows lower fruit production. Other possible way to improve the marolo crop is to enhance natural pollination. In this way it is recommended to reduce the intensity of animal grazing, to preserve natural forests and forest fragments in the vicinity of agroforestry system, and to reintroduce *A. coriacea, A. dioica, A. monticola,* co-occurring species also pollinated by *C. atricapilla,* to enhance natural pollination and fruit set.

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# CAPITULO 5 - IMPLANTAÇÃO DE UM BANCO DE GERMOPLASMA ATIVO *IN SITU* DE *Annona crassiflora* MART. (ANNONACEAE)

# 1 INTRODUÇÃO

As sementes para a produção das mudas foram coletadas em Montes Claros, Curvelo e Morro da Garça, todos no norte de Minas Gerais. Cerca de 400 mudas foram preparadas em saquinhos plásticos de polietileno (30 cm), sendo levadas ao campo após dois anos de semeadura, quando as plantas já haviam atingido aproximadamente 30 cm de altura. Todas as etapas de beneficiamento dos frutos, germinação das sementes e obtenção das mudas de marolo foram realizadas no Viveiro Florestal da UFLA.

A área escolhida para a implantação do banco de germoplasma faz parte da Faz. Caxambu, em Carmo da Cachoeira (21º26'S; 45º05'W), sendo a mesma área de estudo dessa tese. A fisionomia vegetal original da área é o cerrado *stricto sensu,* a topografia é suave sobre latossolo. Esse local foi escolhido pelas seguintes razões: presença de uma população remanescente de marolo na área, disponibilidade e interesse dos proprietários em cuidar das plantas no campo e a facilidade de acesso à área, que dista cerca de 30 km da UFLA.

O preparo do solo foi feito com subsolador (figura 1) e posterior abertura das covas com espaçamento de 8 x 8 m. Foram aplicados 150 g de super simples por cova. As 400 mudas foram misturadas e plantadas aleatoriamente em novembro de 2007 (figuras 2 e 3). As chuvas foram abundantes na estação chuvosa 2007/2008, o que propiciou bom pegamento inicial das mudas. Porém, durante a estação seca de 2008, foi observado alto índice de mortalidade, tendo sobrevivido cerca de 250 mudas. Atualmente o banco de germoplasma possui aproximadamente 400 indivíduos; 150 adultos nativos de Carmo da Cachoeira e 250 mudas provenientes de Curvelo, Morro da Garça e Montes Claros. A condução do plantio está ao encargo dos proprietários da fazenda Caxambu que realizam capinas ao redor das mudas sempre que necessário. O banco de germoplasma será avaliado aos 18 meses, quando serão

obtidos os dados de sobrevivência, altura e diâmetro ao nível do solo de todas as mudas plantadas.



FIGURA 1   Preparo da área com subsolador.



FIGURA 2   Plantio de muda de *Annona crassiflora* em cova adubada com 150g de super-simples e aberta sobre o sulco do subsolador.

FIGURA 3 Muda de *Annona crassiflora* plantada no sulco do subsolador.



FIGURA 4    Imagem de uma das mudas do banco de germoplasma. A foto foi
              tirada em dezembro de 2008.

# 2 CONSIDERAÇÕES FINAIS

Um amplo conhecimento da ecologia de uma espécie é de suma importância para a elaboração de medidas e adoção de práticas para a sua conservação. Entre as questões abordadas nesse estudo estão a dispersão, a fenologia e a polinização em *Annona crassiflora,* uma espécie de importância ecológica e econômica no cerrado. O trabalho permitiu descrever o 'rola bosta' (*Dichotomius ascanius*) como dispersor primário da espécie, sendo este o primeiro relato desses besouros realizando essa tarefa, uma vez que a literatura mundial os considera exclusivamente como dispersores secundários de sementes. O acompanhamento dos eventos fenológicos do marolo por 28 meses permitiu descrever parte do comportamento adaptativo da espécie. O terceiro e último CHAPTER é uma proposta de manejo silvopastoril para o consórcio do marolo com pastagem de brachiaria (*Brachiaria decumbens*).

A implantação do banco de germoplasma de *A. crassiflora*, além de importante para a conservação, servirá como base para o melhoramento genético da espécie.

A diversidade e estrutura genéticas das populações de *A. crassiflora*, que estão sendo geradas a partir de marcadores moleculares, serão analisadas e discutidas comparando os dados ecológicos aqui obtidos. Estudos em escala fina espacial poderão também elucidar aspectos importantes sobre a dispersão da espécie. Outro importante aspecto a ser abordado será o estudo em escala ampla, ao longo da distribuição da espécie. Isto poderá auxiliar a indicar áreas prioritárias para a conservação, com base em dados de diversidade genética. Estes estudos, aliados aos dados aqui apresentados, irão auxiliar no melhor entendimento sobre a ecologia e a genética de *A. crassiflora*.