

## Reproductive biology of *Protium spruceanum* (Burseraceae), a dominant dioecious tree in vegetation corridors in Southeastern Brazil

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**ABSTRACT** – (Reproductive biology of *Protium spruceanum* (Burseraceae), a dominant dioecious tree in vegetation corridors in Southeastern Brazil). We investigated the reproductive biology of *P. spruceanum* (Benth.) Engler in vegetation corridors of secondary Atlantic forest in Lavras, southern Minas Gerais State, Brazil. The reproductive phenology was investigated fortnightly over a one year period. Floral biology studies involved pollen viability analysis, nectar production, stigmatic receptivity, pollen tube growth, visiting insect species and visit rates. The small, pale yellowish flowers (0.3-0.4 cm diameter) are functionally unisexual and organized in dense inflorescences (ca. 45 flowers). *P. spruceanum* presented annual flowering between September and November. Staminate flowers supplied a high percentage of viable pollen (90.6%) and relatively abundant nectar ( $\bar{x}$  = 4.5  $\mu$ L). Pistillate flowers produced only nectar to flower visitors ( $\bar{x}$  = 4.0  $\mu$ L). The effective pollinators were *Apis mellifera* and *Trigona* sp. (Hymenoptera, Apidae). Pollen tubes of cross-pollinated flowers were observed entering the ovaries 48 h after pollination. The fruiting season is from October to March, with a peak in November, coinciding with the rainfall peak. Ecological implications of these findings, and alternative arguments to explain the high genetic diversity at regional landscape are discussed.

Key words - dioecy, floral visitors, flowering, fruiting, secondary forest

**RESUMO** – (Biologia reprodutiva de *Protium spruceanum* (Burseraceae), uma espécie arbórea dioica e dominante em corredores de vegetação no sudeste do Brasil). A fenologia e a biologia reprodutiva de *Protium spruceanum* (Benth.) Engl. foram avaliadas mediante observações quinzenais pelo período de um ano, em corredores de vegetação secundária em Lavras, sul de Minas Gerais. As análises da biologia floral compreenderam a viabilidade polínica, produção de néctar, receptividade estigmática, crescimento do tubo polínico, visitantes florais e taxas de visitas. As flores são pequenas (0,3-0,4 cm de diâmetro), de cor amarela clara, funcionalmente unissexuais e dispostas em densas inflorescências (média de 45 flores). *P. spruceanum* apresentou floração anual e massiva entre setembro e novembro. As flores estaminadas produzem alta porcentagem de pólen viável (90,6%) e quantidade de néctar ( $\bar{x}$  = 4,5  $\mu$ L). Flores pistiladas oferecem apenas néctar para os visitantes ( $\bar{x}$  = 4,0  $\mu$ L). Os polinizadores efetivos são *Apis mellifera* e *Trigona* sp. (Hymenoptera, Apidae). Os tubos polínicos de flores de polinização cruzada foram observados na base do estilete e no ovário após 48 h da polinização. A frutificação ocorre entre outubro e março, com pico em novembro, coincidindo com o período de maior precipitação. São discutidas as implicações ecológicas dos resultados e os argumentos para explicar a alta diversidade genética da espécie na paisagem local.

Palavras-chave - dioecia, floração, floresta secundária, frutificação, visitantes florais

### Introduction

Many members of the Burseraceae family, which are well-known sources of gums and resins (Sunnichan *et al.* 2005), are dioecious and produce small flowers. Information on their reproductive biology however is available for only a few species of this family (Gupta *et*

*al.* 1996, Farwig *et al.* 2004, Sunnichan *et al.* 2005, Voigt *et al.* 2005). *Protium* Burm. f. is a genus of approximately 146 species, occurring mainly in neotropical regions. The primary centre of diversity is in the Amazon rainforest, where 73 species occur, of which 42 are endemic to the region (Daly 1992). *Protium spruceanum* (Benth.) Engler, locally known as *breu*, is a large canopy tree (up to 20 m tall), found in the Amazon and Atlantic rainforests and on the cerrado riverbank woodland (Oliveira Filho & Ratter 1995).

The Brazilian Atlantic forest, in the southern part of the Minas Gerais State, has been seriously exploited since European occupation two centuries ago resulting in the fragmentation and isolation of the forests. At the

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same time, ditches to divide rural properties (*ca.* 4–6 m wide) were constructed by slaves, resulting in vegetation corridors, that is, second-growth colonization by native tree species that connect small fragments of forest (Vieira & Carvalho 2008). In our previous research, a study of *P. spruceanum* throughout the fragments of Brazilian Atlantic forest and corridors of secondary forest revealed that the species exhibited high levels of genetic diversity (Vieira & Carvalho 2008). However, no data about reproductive biology of this species were available. Correlations between genetic variation and reproductive data have been shown to be linked (Crawford & Elisens 2006, Mateu-Andrés & Paco 2006). For this, we investigated the reproductive biology and phenology of *P. spruceanum*, in the Atlantic forest in Lavras, southern Minas Gerais, Brazil.

## Materials and methods

**Study site** – Because trees are concentrated in lower-height reproductive classes rather than in fragments, this study was conducted in vegetation corridors, located in the city of Lavras, in southern Minas Gerais State, Brazil (21°17'33" S and 44°59'15" W, 21°18'11" S and 44°59'18" W). The region is characterized by a Köppen type Cwa climate, with a rainy summer and a dry winter season, with mean annual rainfall of approximately 1,500 mm and mean annual temperature of approximately 20 °C. The current landscape comprises a number of forest fragments and a matrix of planted pastures and vegetation corridors of secondary forest (Vieira & Carvalho 2008). Fragments and vegetation corridors are similar in terms of floristic composition, but corridors have larger basal areas and trees are concentrated in the upper diameter classes and lower height classes. In the corridors studied, *Protium spruceanum*, along with companion tree species such as *Tapirira guianensis* Aublet (Anacardiaceae), *Copaifera langsdorffii* Desf. (Fabaceae) and *Ocotea pulchella* Mart. (Lauraceae) are the most abundant species (> 120 trees ha<sup>-1</sup>) of the mass-flowering/insect-pollinated and bird-dispersed tree species. The absolute density of *P. spruceanum* in these corridors was estimated as 135 trees ha<sup>-1</sup> (G.C. Castro, unpublished data).

**Phenological observations** – Phenological observations for all reproductive trees were made fortnightly from November 2005 to December 2006, recording initial and final phenological periods. Absence or presence of buds, flowers and fruits were recorded in four vegetation corridors (*ca.* 2,650 m length). Flowering (anthesis) and fruiting (green and ripe fruit) were recorded as the presence of one or more open flowers or one or more fruit, respectively. Rainfall and temperature data were available during phenological observations from the Estação Meteorológica da Universidade Federal de Lavras (approximately 8 km north of study site). Number of flowers

at anthesis per inflorescence was estimated by counting the number of flowers in seven trees (2–4 inflorescences per tree).

**Floral biology** – Nectar volume and sugar concentration were measured on 61 bagged flowers from six trees, using graduated micro-capillaries and a hand refractometer. The volume and concentration of the accumulated nectar were estimated at approximately 08h00, when anthesis had started. Pollen viability was estimated in 13 male flowers, using the acetocarmine staining technique, under a microscope (Kearns & Inouye 1993). Stigmatic receptivity was determined through the peroxidase activity technique (Kearns & Inouye 1993), between 08h00 and 15h00, in both sexual morphs ( $n = 69$ , from six trees).

**Pollen tube growth** – Pollinated flowers resulting from cross-pollinations were collected, the pistils removed, and fixed with FAA for 24 hours. Following this, they were transferred to ethanol (70%) and then stored at 8 °C. Pollen tube growth was investigated in pistils fixed 24 and 48 h after hand pollination. The pistils were softened for 30 min in NaOH 6 M in a water bath (60 °C) and then rinsed in water. The pistils were stained with 0.1% aniline blue for 4 h, squashed under a cover-slip, and observed under a fluorescence microscope (Olympus BX 60, USA).

**Floral visitors** – Floral visitor activity was recorded during two days of field observations, usually from 07h00 to 16h00, during massive flowering. Three observers working separately enabled approximately 54 hours of observations to be recorded. The resource gathering and movements regarding contact with stigma were recorded for all visitors. At least two individuals of each visitor morphospecies were captured for identification. The frequency curves of floral visitor were compared by Kolmogorov-Smirnov's nonparametric test.

## Results

*Protium spruceanum* presented massive annual flowering, between September and November. Overall reproductive activity was greatest in the rainy season and lower or absent in the dry season. Floral buds started to develop in July, in the transition period from dry to wet season. Flowering commenced with the beginning of the rains. The trees had dense inflorescences (median = 45 flowers, range = 15–87,  $N = 22$ ). The species is cryptically dioecious, with apparently perfect flowers possessing either rudimentary gynoeceum lacking ovules (male plant) or vestigial anthers with no pollen (female plant). Anthesis started in the early morning and flower opening occurred during the day for flowers of both sexes. Staminate flowers supplied a high percentage of viable pollen ( $90.6\% \pm 1.2$  s.e.,  $n = 13$  flowers) coinciding with the receptive phase of the stigma of the pistillate

flowers that started immediately after flowering and extended throughout the day. Staminate and pistillate flowers produced relatively abundant nectar (4.5 and 4.0  $\mu\text{L}$ , respectively), with an average of 28.3% and 32% concentration in sucrose equivalents, respectively.

The flowers of *P. spruceanum* were visited by a low total number of insect species, including bees, wasps, flies and small ants. The bees *Apis mellifera* and *Trigona* sp. (Hymenoptera, Apidae) were the most frequent visiting species (figure 1), probably due to the high number of flowers/inflorescence of *P. spruceanum* in the corridors of secondary forest. *Trigona* sp. individuals remained for several minutes foraging on a single flower. They remained most of the time in a single plant, compared to *A. mellifera*, that restricted their visits to less than a minute per flower and foraged a few flowers per plant (personal observation). Agonistic interespecific encounters were not common. The flowers of *P. spruceanum* were visited mainly between 09h00-12h00 when most of the floral visitors contacted anthers and stigma. The difference in the frequency of visits between *Apis mellifera* and *Trigona* sp. was not significant ( $P = 0.090$ ,  $\alpha = 0.05$  by Kolmogorov-Smirnov's test) (figure 1).

Cross-pollen on pistils germinate and tubes grow down the pistils for up to 24 h. Pollen tubes of cross-pollinated flowers were observed at the base of the pistils until 48 h after pollination. The medium-sized, bird-dispersed seeds (< 500 mg fresh weight) are produced in reddish berries in the canopy of adult trees and are dispersed from October to March, with a peak in November, coincidentally when the rainfall peaks. The fruits have an outer covering that splits in half when mature, exposing between one and three grey seeds enveloped by a fleshy white aril. The fruits are dispersed by birds.

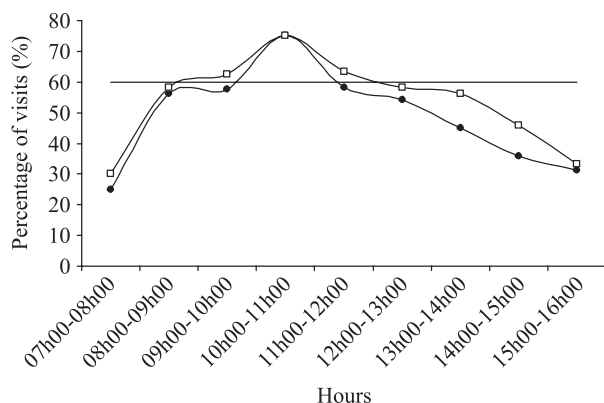


Figure 1. Frequency of visits of *Apis mellifera* (□) and *Trigona* sp. (●) on flowers of *Protium spruceanum* during the monitored hours. The line in the percentage 60 detaches the periods of high floral visits (> 60%).

## Discussion

The phenological pattern in our study showed a trend towards a concentration of flowering and fruiting of *P. spruceanum* in the early wet seasons at the warmest period of the year. This is in accordance with the general findings in the tropics, particularly where there is climatic seasonality (Schaik *et al.* 1993). Likewise, in Atlantic forest the flowering is, in general, highly seasonal and concentrated at the beginning of the wetter season (Morellato *et al.* 2000). This might be a consequence of marked irregularity in rainfall distribution and amount, that could be expected to lead to irregularity in flowering for certain species. The development of ripe fruits at the beginning of the warm and wet season is likely to be adaptive, so that germination and establishment can take place while water is plentiful (Schaik *et al.* 1993). Indeed, visual observations in the field indicate that germination of seeds and *P. spruceanum* seedling emergence occur in the rainy season.

Spatial patterns of genetic variation within populations depend primarily on the patterns and distance of pollen and outcrossing rates and these results have often been interpreted as the consequence of intense gene flow (Doligez & Joly 1997, Streiff *et al.* 1998). Two of the most observed visitors of dense inflorescences of *P. spruceanum* are *Apis mellifera* and *Trigona* sp., and the opportunistic behavior of such family (Apidae) has been reported in many habitats and associated with plant density and clusters (Ramalho 1990). Though exotic honeybees can be important competitors of native pollinators (Carmo *et al.* 2004), sometimes they have neutral or even beneficial effects on the pollination of native plants (Gross 2001, Dick *et al.* 2003, Suzuki 2003), because of their high density, social organization and propensity for agricultural landscapes (Dick *et al.* 2003). High temperatures around midday could result in high insect activity, as observed in this study, and this could lead to high visiting rates (Arroyo *et al.* 1985).

*Apis mellifera* and *Trigona* sp. bees were considered efficient pollinators due to their frequency of visits at the flowers and also because they could easily contact anthers and stigmas. This is consistent with other studies with bees being the most important pollinators for other tropical tree species of the family Burseraceae (Bawa 1990, Farwig *et al.* 2004, Voigt *et al.* 2005). In a review about outcrossing and pollen-mediated gene flow in neotropical trees, Ward *et al.* (2005) found no mating system studies (*e.g.* paternity analysis) for species of Burseraceae. However, Dunphy & Hamrick (2007) found



long-distance pollen movement, combined with almost total outcrossing, which was likely to be responsible for the low levels of population divergence and the relatively high genetic diversity for the neotropical tree *Bursera simaruba* (Burseraceae).

Low genetic differentiation among forest fragments and vegetation corridors of secondary forest was observed in a previous allozyme study of *P. spruceanum* (Vieira & Carvalho 2008). Male and female trees can differ in their attractiveness to pollinators; staminate flowers provide pollen and mostly nectar, whereas pistillate flowers often have only nectar (Bawa 1990). Consequently, the main advantage of dioecy may be avoidance of selfing. This might result in more fruit set in comparison with monoecious and hermaphroditic plant species (73.8% vs. 42.1%) (Sutherland & Delph 1984). Indeed, vegetation corridors presented high gene diversity ( $H_e = 0.420$ ), and absence of inbreeding (Vieira & Carvalho 2008). Nevertheless, further studies of sex ratio variation, spatial distribution and parentage analysis (Dunphy & Hamrick 2007, Pavón & Ramírez 2008) across the vegetation corridors are necessary to provide a clear picture of the contribution of seed and pollen to the overall contemporary gene immigration. The comparison between historical estimates of gene flow using variance in allelic frequencies, and contemporary estimates of gene flow using parentage assignment or reproductive biology data is expected to provide insights into ecological and evolutionary processes at regional landscape within and among populations.

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