

## Morphological characterization of fruits and *in vitro* germination of *Byrsonima ligustrifolia* A. Juss. (Malpighiaceae)

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### Workshop Information

I Workshop of Plant Biology (I Workshop de Biologia Vegetal) was held in the Bioscience Institute – UNESP, campus of Rio Claro, Brazil, during August 20 and 21, 2012. Workshop was a scientific event organized by Post-graduate students from that Institute aiming to integrate Post-graduate and Graduate students from different areas related to Plant Biology (Anatomy, Ecology, Evolution, Morphology, Physiology, and transitional areas) from different Universities. Workshop Organization offered a large number of speaking activities, scientific discussions, and extra short-courses to improve the knowledge and formation of students in Plant Biology.

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### INTRODUCTION

The family Malpighiaceae has a tropical and subtropical distribution (Souza and Lorenzi 2008), although it is especially diversified in South America (Judd et al. 2009). The family includes 75 genera and approximately 1300 species; 45 genera and approximately 300 species occur in Brazil (Souza and Lorenzi 2008).

In Brazil, species of the genus *Byrsonima* are found from Amazonia to Paraná, with approximately 60 species that are popularly known as “Murici.” Almeida et al. (1998) remarked that plants belonging to this genus are also known for their high nutritional and medicinal value. According to Lorenzi (2002), members of this genus show a low germination rate and slow seedling emergence, which make sexual propagation difficult.

The scientific literature on Malpighiaceae is rather sparse. Sparser yet are studies on the species *Byrsonima ligustrifolia* A. Juss., which occurs abundantly in restinga areas in Cananéia, a municipality located in the Vale do Ribeira, state of São Paulo, Brazil. The Cananéia region possesses

one of the largest remnants of the Atlantic Rainforest. *Byrsonima ligustrifolia*, which in this region is popularly known as “Muchita,” is highly esteemed by the local population for its edible fruits, and also shows potential for use in urban reforestation; these virtues lend importance to the study of its germination and morphological analysis of the fruits.

### MATERIAL AND METHODS

**Plant material.** Fruits were collected from individuals of *B. ligustrifolia* in the Atlantic Rainforest Restinga area of the municipality of Cananéia, São Paulo, Brazil (25° 01' 35" S, 45° 57' 43" W).

**Morphological description of the fruits.** The fruits were morphologically characterized based on the following parameters: definition of the coloration, according to visual analysis based on the scale of the degree of maturation; and measurements of mass, height, and diameter of the fruits, with and without pulp (100 fruits analyzed); number of seeds per fruit; and number of fruits predated (180 fruits analyzed). The height and diameter were measured with a

caliper. The mass was measured on a precision balance.

**Culture media.** Two culture media were used: Water-Agar Medium and MS½ Medium. The Water-Agar Medium was made from 0.6% agar dissolved in distilled water and sterilized in an autoclave (125 °C) for 20 min. The MS½ Medium corresponds to MS medium (Murashige and Skoog 1962) modified to contain half the originally described amount of macronutrients. The MS½ Medium was prepared with salts (M0153) and vitamins (1000X concentrated solution) obtained from Sigma and used according to the manufacturer's instructions. This medium also contained 2% saccharose and 0.6% agar. The pH was adjusted to 5.7 and the medium was sterilized in an autoclave (125 °C) for 20 min.

**Disinfestation of depulped fruits and culture conditions *in vitro*.** The depulped fruits were treated with absolute ethanol for 1 min. The ethanol was then removed and replaced with an aqueous solution of 50% bleach (1.0-1.25% sodium hypochlorite) and 0.1% Tween20 detergent. The fruits remained in contact with this solution for 20 min, after which the solution was discarded and the fruits were washed three times in distilled water. Next, the fruits were treated with a 1% mercury chloride solution for 5 min and then washed three times in distilled water (Santos and Wendling 2010). The fruits were placed in the culture flasks with the aid of tweezers, with four fruits per flask; all the work was conducted in a laminar-airflow chamber and using sterilized materials. For each culture medium, 24 flasks were used (totaling 96 depulped fruits); and for each condition, half of the bottles were kept in a constant photoperiod of 16 hours light, and half were kept in the dark for 10 days and then exposed to a photoperiod of 16 hours light. The material was maintained at a temperature of 25 °C and 70% relative humidity, and evaluated for 100 days.

## RESULTS AND DISCUSSION

*Byrsonima ligustrifolia* has fleshy fruit containing a drupe with three locules, and may contain up to three seeds. The calyx has persistent oil glands. The coloration of the fruit changes during the maturation process from green to red, vinaceous, and then purple at the peak of maturity (Figure 1A). The fruit varies from spherical to oval, with the height ranging from 0.82 to 1.09 cm (mean 0.99 cm; standard deviation 0.06 cm) and diameter D1 (1.02 to 1.54 cm) similar to D2 (1.03 to 1.52 cm), both with the same mean (1.26 cm) and standard deviation (0.09 cm). The fresh weight of the fruit varied from 0.65 to 1.70 g (mean

1.03 g; standard deviation 0.21 g). The core, with the same shape, ranges in height from 0.53 to 0.66 cm (mean 0.58 cm; standard deviation 0.03 cm), diameter from 0.50 to 0.70 cm (mean 0.60 cm; standard deviation 0.04 cm), and weight from 0.07 to 0.17 g (mean 0.11 g; standard deviation 0.02 g) (Figure 1B), and comprises only 10.7% of mean weight of fruits.

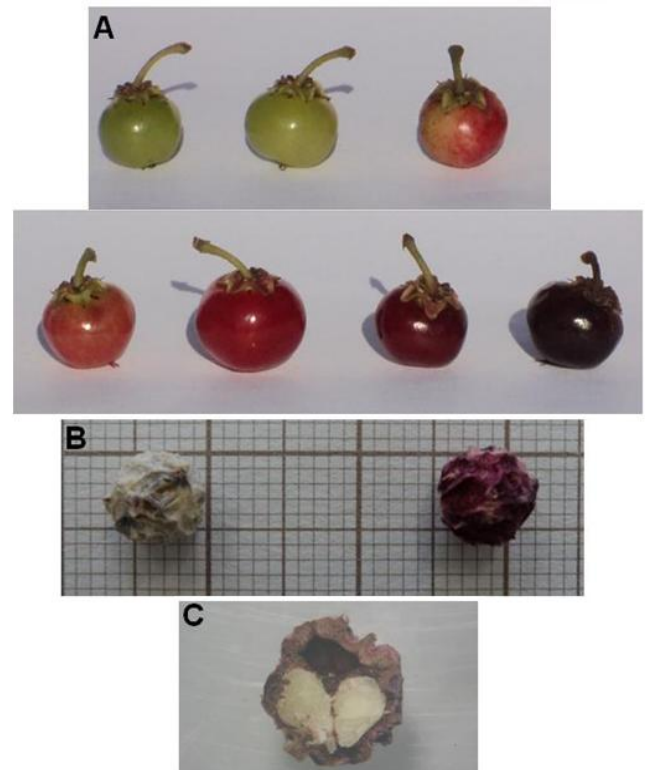


Figure 1. Morphology of fruits of *B. ligustrifolia*. A) Coloration of fruits with pulp, based on the degree of maturation – from green to mature fruit. B) Detail of two depulped fruits. C) Detail of an opened depulped fruit with one empty locule (two seeds present).

Because of the low germination rate and slow seedling emergence observed for members of the genus (Lorenzi 2002), it was decided to test the viability of germination *in vitro*, with two different culture media and different light regimes. However, after 100 days of observation, no signs of germination (rootlet emergence) were observed under the conditions tested.

Based on these results, and considering that the fruits were found to be predated by an unidentified type of insect larva, it was decided to examine other parameters, including the number of seeds per fruit and the number of fruits predated. Of the sample analyzed, 69.44% of the fruits were intact, i.e., not predated; and 30.56% had at least one record of predation, to a maximum of three per fruit. However, regardless of whether or not the fruits were predated, the majority of the cores (62.04%) contained only one seed, with a maximum of three (Figure 1C).

These results suggest that the germination inefficiency is not related to the presence of the

predator, but to the low number of seeds and possibly the occurrence of dormancy, a natural mechanism in which the plant rests for a period (Baskin and Baskin 2000). Dormancy may be related to unfavorable environmental conditions or to morphophysiological factors of the species (Alves et al. 2000, Nunes et al. 2006).

## CONCLUSIONS

Studies on morphological characterization and germination are of great value to improve knowledge of species, mainly to support cultivation strategies aiming for conservation and also production of seedlings. The results obtained here are new for the species under study, *B. ligustrifolia*. The preliminary characterization of the fruit revealed even more clearly its potential for food, since most of the fruit consists of the fleshy edible pulp (the core comprises only 10.7% of the fruit). Also notable was the low number of seeds per core, and that the predation on the fruit was not directly related to successful germination. This analysis makes clear the need to establish new investigative methodologies, such as the use of techniques for breaking dormancy.

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