

# Physiological dormancy and seed germination inhibitors in *Miconia* (Melastomataceae)

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**Background and aims** – Seed dormancy is rarely studied in the Neotropical area preventing attempts to understand its phylogenetic and biogeographic distribution.

**Methods** – We estimated seed viability and germination of fresh-collected seeds of *Miconia ligustroides* and *Miconia pepericarpa*. Seed coat permeability tests and embryo anatomy analyses were performed to determine seed dormancy class. The effect of different concentrations of extract of mature berries on seed germination was experimentally tested.

**Key results** – Both species produce dormant seeds with permeable seed coats and differentiated embryos, thus seeds are physiologically dormant. Both species disperse seeds when conditions for seedling establishment are not suitable, thus dormancy is considered a strategy that may have evolved as a response to drought-induced seedling mortality. Fruit extracts of *M. ligustroides* inhibited lettuce seed germination, suggesting that seed cleaning by frugivores is required prior to seed germination.

**Conclusions** – Seed dormancy in both species should decrease seedling mortality at the onset of the dry season and delay germination to the following rainy season, when conditions for seedling establishment are suitable. Germination inhibitors in *Miconia* fruits indicate that seed cleaning is an important contribution to plant fitness delivered by frugivores.

**Key words** – convergent evolution, frugivory, germination inhibitors, physiological dormancy, seasonality, seed cleaning, rupestrian grasslands.

## INTRODUCTION

The evolution of seed dormancy was a remarkable event in the life history of seed plants (Linkies et al. 2010). Definitions of seed dormancy are often disputed in the literature (see Thompson & Ooi 2012 and references therein), but here we follow the definition by Hilhorst (2011): seed dormancy is the absence of germination of a viable seed under conditions that are favourable to its germination. Because germination is an irreversible process, selection should favour strategies that prevent seeds from germinating under unfavourable conditions for seedling establishment and survival (Jurado & Moles 2002).

Seed dormancy is rarely studied in Neotropical regions, preventing us from fully understand its phylogenetic and biogeographic distribution (Baskin & Baskin 2005, Silveira 2013). Here, we report the occurrence of seed dormancy in two *Miconia* (Melastomataceae) species from Neotropical montane vegetation (rupestrian grasslands). Melastomataceae is a species-rich family occurring in both aseasonal and seasonal vegetations in South America (Clausing & Renner

2001, Goldenberg et al. 2008). Species in the aseasonal forests lack seed dormancy whereas species growing in seasonal savannas often present physiological dormancy (Silveira et al. 2013). This fact suggests a major role of seasonality in the evolution of seed dormancy. In seasonal vegetations, however, dormancy evolved only in species shedding seeds under unfavourable conditions for seedling establishment (i.e. at the end of the rainy season) (Silveira et al. 2012a). Because environmental conditions are favourable for seed germination but not for seedling establishment and survival at the end of the rainy season, seed dormancy in Melastomataceae is interpreted as a strategy to decrease seedling mortality during the dry season (Silveira et al. 2012a).

Here, we studied seed dormancy in *Miconia pepericarpa* DC. and *Miconia ligustroides* Naudin. These species were selected for this study because both fruit at the end of the rainy season, thus matching the conditions under which seed dormancy has evolved in Melastomataceae (Silveira et al. 2012a). We have also investigated the effects of fruit extracts of *M. ligustroides* on seed germination and seedling devel-

opment. There are few reports of germination inhibitors in *Miconia* berries from forest species (Silveira et al. 2013), but to the best of our knowledge there are no published papers for seasonal vegetations.

## MATERIAL AND METHODS

Berries of *M. ligustroides* and *M. pepericarpa* were collected from 5–40 individuals at Serra do Gandarela (20°08'17"S 43°41'22"W) and Lavras Novas (20°28'38"S 43°33'19"W), in May and June 2012, respectively. Vouchers are deposited at BHCB (UFMG). Seeds of both species were collected in the species-rich vegetation known as rupestrian grasslands (rocky montane grasslands) in Minas Gerais, southeastern Brazil. This vegetation occurs in quartzite-derived or ironstone soils above 900 m above sea level and harbour high levels of plant diversity and endemism (Alves & Kolbek 2010). Plant communities establish under very stressful conditions including shallow, acidic, nutrient-poor, and excessively drained soils, strong winds, frequent fires, high daily thermal amplitudes and water shortage during the dry season (Giulietti et al. 1997, Jacobi & Carmo 2011). Both *Miconia* species colonise rocky outcrops gravels and sandy soils with low water retention capacity. The climate in the study sites is seasonal with rainy summers (from October to April) and dry winters from May to September.

Experiments were done immediately after seed collection following a standardised protocol (Silveira 2013). Seeds were manually extracted from fruits, washed in tap water for 5 minutes and dried under shade for 24 h. Seed viability was assessed by seed dissection and by means of the tetrazolium test. Five replicates of 20 seeds for each species were cut under a stereomicroscope and checked for embryo presence/absence. Afterwards, cut seeds containing embryos were soaked in a 1% solution of tetrazolium in darkness at 25°C for 24 h. To estimate total germinability, five replicates of 20 seeds were set to germinate in 9-mm Petri dishes on two sheets of filter paper moistened with Nistatin solution (2%) to prevent fungi growth. The Petri dishes were incubated under optimum conditions for melastome seeds (Silveira et al. 2013): constant temperature of 25°C at a 12:12 hour light:dark cycle. The trials were monitored at 24-h intervals for 30 days, and seeds were considered germinated when radicle emergence was observed. This 30-day interval is considered enough to discriminate between dormant from non-dormant seeds (Baskin & Baskin 2004, 2005).

For each treatment, we calculated final germinability (%), mean germination time (MGT) and germination synchrony ( $\bar{E}$ ) (Ranal & Santana 2006):

**Table 1 – Mean (±SE) percentage of embryoless seeds, viable embryos, polyembryony and germination traits of two *Miconia* species from Neotropical montane savannas in Southeastern Brazil.**

Species	Seeds with embryos (%)	Seed viability (%)	Polyembryony (%)	Germinability (%)	Mean germination time (days)	Synchrony (bits)
<i>M. pepericarpa</i>	82.0 ± 2.6	70.0 ± 4.7	8	30.0 ± 5.7	22.8 ± 1.1	1.1 ± 0.3
<i>M. ligustroides</i>	87.2 ± 3.9	14.4 ± 5	0	5.6 ± 1.6	29.0 ± 0.6	0

$$\text{MGT} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

where  $n_i$  is the number of seeds germinated at time  $i$ ,  $t_i$  is the time from the start of the experiment to the  $i^{\text{th}}$  observation and  $k$  is the time of last germination.

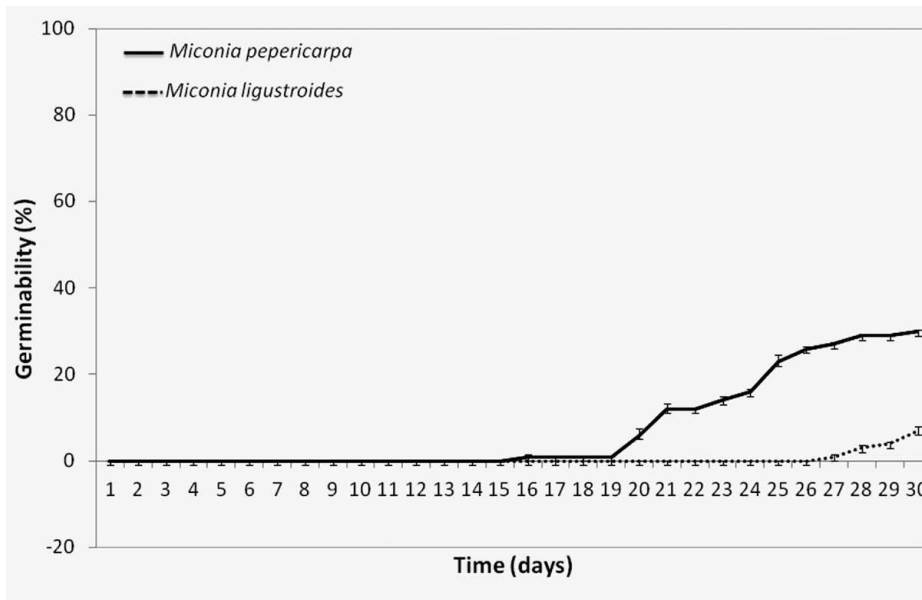
$$\bar{E} = - \sum_{i=1}^k f_i \log_2 f_i \text{ being } f_i = n_i / \sum_{i=1}^k n_i$$

where  $f_i$  is the relative frequency of germination,  $n_i$  is the number of seeds germinated at time  $i$  and  $k$  is the time of last germination. High  $\bar{E}$  values indicate asynchronous germination. We have also calculated the percentage of polyembryonic seeds (Mendes-Rodrigues & Oliveira 2012).

To determine seed coat permeability, five replicates of 20 fresh seeds for each species were weighed on a digital scale. Seeds were soaked in tap water for 72 h at room temperature and reweighed. Seed permeability was determined by the increase in seed mass and differences in percentage increase between seed mass of dried and soaked seeds were determined by Wilcoxon test (Silveira 2013).

To examine embryo anatomy, seeds were fixed in formalin-acetic acid-50% ethanol (Johansen 1940) for 48 h, with a 24-h vacuum passage. Seeds were kept in Franklin solution for 8 h at 60°C until clearing (Franklin 1945, modified) and samples were rinsed with distilled water (see Silveira et al. 2012a; supplementary material). The seeds were dehydrated in ethanol series, infiltrated in 2(hydroxy-ethyl)-methacrylate (Leica™) and in the embedding solution (Paiva et al. 2011). The longitudinal and transversal 7-µm-thick sections were prepared in a rotary microtome (Zeiss Hyrax M40), stained with toluidine blue 0.05%, pH 4.7 (O'Brien et al. 1964, modified) and mounted in Entellan™. The putative mechanism accounting for physiological dormancy in melastomes is the occurrence of phenolics on their seed coats (Silveira et al. 2012a). To detect phenolics in the seed coats, 8-µm sections were stained with 10% aqueous ferric chloride plus sodium carbonate (Johansen 1940).

To examine whether the fruit extracts of *M. ligustroides* contain germination inhibitors, 90 fruits were mashed in 40 ml of distilled water. Three treatments were conducted: control (distilled water), 50% extract and 5% extract. Lettuce seeds (5 replicates of 20 seeds per treatment) were set to germinate under Petri dishes incubated at a 12-h photoperiod under constant temperature of 25°C. After seven days we recorded final germinability and the number of normally developed seedlings. The assumptions of the parametric analyses were checked prior to analyses and arcsine transformations were employed whenever necessary (Sileshi 2012). Data on germinability were compared with Mann-Whitney's U and



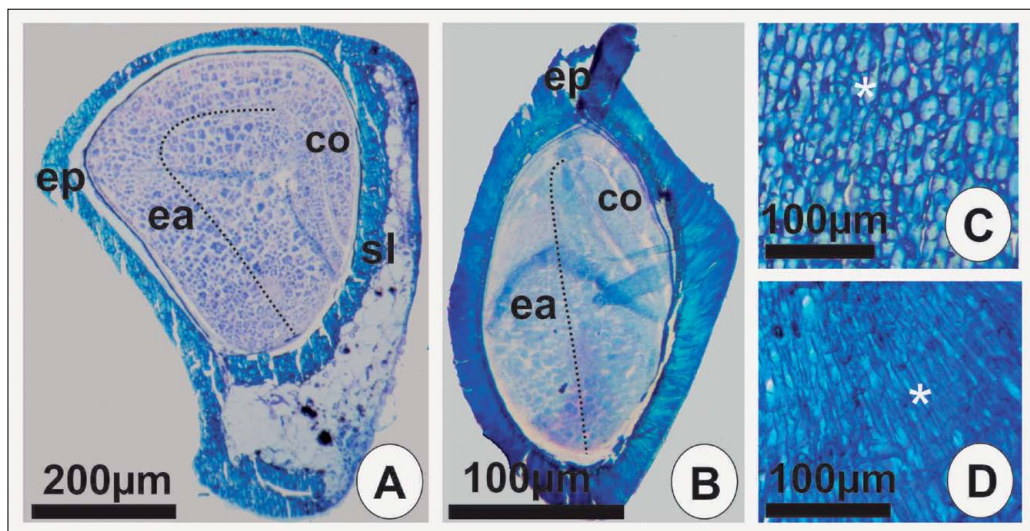
**Figure 1** – Cumulative germinability (%) of seeds from *Miconia ligustroides* and *Miconia pepericarpa* (Melastomataceae) collected in southeastern Brazil. Bars represent standard deviation.

data on seedling development were compared with two-sample t-tests. For all analyses we used  $\alpha = 0.05$ .

### RESULTS

The percentage of seeds containing embryos was higher than 80% for both species (table 1). Most of these embryos were viable, but germination percentages were only 30 and 6% for *M. pepericarpa* and *M. ligustroides*, respectively (fig. 1). Seed viability was low for *M. ligustroides*, and most viable seeds (61%) did not germinate. Moreover, germination of both species was low and asynchronous (table 1). *M. pepericarpa* produced polyembryonic seeds and *M. ligustroides* produce only monoembryonic seeds (table 1).

Seeds of both species are small and have low fresh dry mass (0.018 mg for *M. pepericarpa* and 0.0061 mg for *M. ligustroides*; average of 20 seeds). After a 72-h imbibition period we found a 64.4 and 61.3% increase for *M. pepericarpa* and *M. ligustroides*, respectively. This increase was significant for both species ( $U = 15$ ;  $p < 0.05$ ). Mature seeds are exarillate and exalbuminous. The embryos are well-developed, showing a conspicuous, differentiated embryo axis and two fleshy cotyledons. The embryos are of the total type, occupying the whole seed, and folded (*sensu* Martin's 1946 classification) with cotyledons parallel to the embryo axis (fig. 2A–B). The epidermis in the seed coat of both species is multiseriate. This epidermis coat contains dead cells with walls that are homogeneous and thick (fig. 2A–B). Phenol-



**Figure 2** – Longitudinal (A–B) and transversal (C–D) sections of dormant seeds of *Miconia* species. A & C, *Miconia ligustroides*; B & D, *Miconia pepericarpa* (co, cotyledon; ea, embryo axis; ep, epidermis; sl, sclerotic layer; asterisk, phenolic compounds).

ic compounds were observed in cell walls in all seed coats (fig. 2C–D).

Fruit extracts of *M. ligustroides* strongly inhibited germination of lettuce seeds. At 50% concentration, extracts completely inhibited germination. At 5% concentration, there was a moderate and significant ( $U = 15$ ;  $p = 0.008$ ) decrease in germination (16% compared to control), but only 5% of the seedlings developed normally compared to controlled conditions ( $t = 23.75$ ;  $p < 0.001$ ). Abnormal seedlings produced atrophied cotyledons, lacked at least one leaf and showed extensive signs of necrosis in the roots.

## DISCUSSION

Seeds of *M. pepericarpa* and *M. ligustroides* contain viable embryos that do not germinate under favourable conditions for its germination. For both species a large fraction of the viable seeds did not germinate under favourable conditions and therefore, non-germinating seeds are considered to be dormant (Hilhorst 2011, Silveira 2013). However, dormancy is not an all or nothing trait (Baskin & Baskin 2004), so the few germinating seeds of both species are considered to be non-dormant. The seed coats of both species are water-permeable and enclose well-developed and differentiated embryos. These two traits indicate that seeds of both species are physiologically dormant (Baskin & Baskin 2005). Chaves et al. (2011) recently suggested that embryos of *M. ligustroides* are undifferentiated. Our data contrasts with their study as we clearly show fully differentiated embryos for this species. Our data also differs from that of Mendes-Rodrigues & Oliveira (2012) who have shown polyembryonic seeds for *M. ligustroides* and monoembryonic seeds for *M. pepericarpa*.

Our data is in accordance with the hypothesis that PD (physiological dormancy) evolved in species dispersing seeds at unfavourable sites and seasons for seedling establishment and survival (Silveira et al. 2012a). Both populations occur in xeric microhabitats and disperse seeds at the transition between the rainy and the dry season, when soil water is enough to trigger germination but insufficient to allow seedling establishment. Under these conditions, the likelihood of seedling mortality is high (Silveira et al. 2012a), and PD appears to have evolved in response to high seedling mortality. If germinated at the transition between rainy and dry seasons, the small-sized seedlings of *Miconia* species would have to survive a forthcoming 5-month dry period before the arrival of perennial rains in October. Mechanisms accounting for breaking PD in both *Miconia* are currently unknown, but we might expect seeds to germinate at the onset of the following rainy season, when the likelihood of establishment and survival is greater.

Physiological dormancy is the most common type of dormancy in Angiosperms and across vegetations types in Earth (Baskin & Baskin 2005). The gain and loss of PD occurred many times during the evolution of seed plants (Linkies et al. 2010), including the Melastomataceae (Silveira et al. 2012a). PD may have evolved independently in both *Miconia* species because these two species are phylogenetically distant within the genus (Goldenberg et al. 2008). The independent evolution of PD in *M. pepericarpa* and *M. ligustroides* adds to new records of PD in Neotropical Melastomataceae (see also

Baskin et al. 1999) and contributes to the understanding of seed dormancy in Neotropical montane vegetation (Baskin & Baskin 2005).

Our data indicate that berries of *M. ligustroides* contain germination inhibitors that are effective in inhibiting germination and cause seedling deformities even in low concentrations. Despite the unknown chemical nature of germination-inhibiting compounds, they appear to be widespread in *Miconia* species (Silveira et al. 2013). *Miconia* berries are primarily dispersed by birds and fruits fallen to the ground are secondarily dispersed by ants (Silveira et al. 2012b, Lima et al. 2013). Seeds of *Miconia* species do not germinate within intact fruits, indicating the germination inhibitors in the fruits may account for the inhibitory effect (Silveira et al. 2013). Since seeds within intact fruits are unable to germinate, seed cleaning can be considered an ecologically important benefit that plants derive from interactions with birds and ants (Silveira et al. 2012b, Lima et al. 2013).

In summary, we have presented evidence for the presence of seed dormancy in populations of *Miconia pepericarpa* and *Miconia ligustroides*. PD in both species should decrease drought-induced seedling mortality at the beginning of the dry season and delay germination and subsequent establishment to the following rainy season, when conditions for seedling establishment are suitable. We also have shown that there are germination inhibitors in the fruits of *M. ligustroides*, indicating that seed cleaning is an important contribution to plant fitness delivered by frugivores.

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