Silva Lusitana, nº Especial 77 - 90, 2014

Propagation of the Azorean native *Morella faya* (Aiton) Wilbur

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Abstract. The aim of this work was to produce *Morella faya* (Aiton) Wilbur plants for Azorean wildlife habitat and conservation landscaping. With that purpose we performed several germination and cutting trials, and measured the plantlets development on different substrates. In the germination trials we tested the effect of chemical scarification of seeds, stratification and the effect of temperature and photoperiod on their germination characteristics. In the cuttings trials, we used semi-hardwood cuttings harvested in October and planted in substrate and softwood cuttings harvested in April and placed in aeroponic conditions, to test the effect of (0 and 0.4%) indole butyric acid on rooting. Mortality of cuttings was 100%. After 34 weeks, fruits' scarification under a suitable light and temperature regime enhanced the percentage of germination. The best regimes of temperature and light were: 15ºC/8h or environmental conditions (starting in October), resulting respectively in 23% and 22,5% germination, 134 and 126 days of mean time of germination, and 82 and 72 days for the first radicle emergence. Survival of the produced plants was superior (95%) when using the soil from the plant's habitat but plant development was superior on the mixture: BVB (NPK): perlite (2:1).
Key words: Azores, cuttings, germination, *Morella faya*, wildlife habitat, landscaping conservation

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Propagação de *Morella faya* (Aiton) Wilbur, uma espécie nativa dos Açores
Sumário. Este trabalho teve como objectivo produzir exemplares de *Morella faya* (Aiton) Wilbur para a conservação de habitats naturais e recuperação paisagística, tendo sido realizados ensaios de germinação, estacaria e desenvolvimento de plântulas. Nos ensaios de germinação testaram-se os efeitos da escarificação química, da estratificação e dos regimes de temperatura e luz nas características da germinação. Nos ensaios de estacaria, testou-se o efeito do ácido indol-butírico (0 e 0,4%) em estacas semi-lenhosas colhidas em Outubro e plantadas em substrato e em estacas verdes colhidas em Abril em condições aeropónicas. Nas condições testadas foram obtidas novas plantas apenas por semente. Após 34 semanas a escarificação dos frutos associada a um regime de luz e temperatura favorável aumentou as percentagens de germinação. Os melhores regimes de temperatura e luz foram: 15ºC/8h e condições ambientais (para ensaios iniciados em Outubro), resultando respectivamente em 23% e 22,5% de germinação, 134 e 126 dias para o tempo médio de germinação e 82 e 72 dias para o período de latência. Após 3 meses a sobrevivência das plântulas foi superior em terra (95%), mas a altura das plantas sobreviventes e o número de folhas por planta foi maior no substrato comercial adubado: BVB (NPK): perlite (2:1).
Palavras-chave: Açores, estacaria, germinação, habitats, *Morella faya*, paisagem, recuperação

Propagation du *Morella faya* (Aiton) Wilbur, une espèce natif des Açores
Résumé. Cette travail a eu comme objectif produire des plantes de *Morella faya* (Aiton) Wilbur pour la conservation des habitats naturelles y la récupération des paysages en exécutant des essais de germination, bouturage et développement des plantules. On a testé les effets da la scarification chimique et du régime de lumière et température sur les caractéristiques de la germination des semences et l'effet de l'acide indol-butirique (0 e 0,4%) sur l'enracinement des boutures simples aoûtées (récoltés en Octobre, sur le substrat) et herbacées (récoltés en Avril, en aéroponie). Pour les conditions testées, on a obtenu plantes seulement par semence. Après 34 semaines les pourcentages de germination on été supérieurs pour fruits avec scarification aux régimes de lumière et température de 15ºC/8h ou les conditions environnementales (pour des essais initié en Octobre), avec respectivement 23% e 22,5% de germination, 134 et 126 jours pour le temps moyenne de germination et 82 et 72 jours pour que la premier semence germiné dans chaque essais. Après trois mois de culture la survivance des plantules sur son substrat naturelle a été supérieur (95%) mais la taille et le numéro des feuilles pour plant a été supérieur sur le substrat commercial amendée BVB (NPK): perlite (2:1).
Mots clés: bouturage, germination, habitats, Les Açores, *Morella faya*, paysage, récupération

Introduction

Morella faya (Aiton) Wilbur belongs to the family *Myricaceae*. This family includes three genera and around 50 species world spread, generally in temperate zones or subtropical regions (CRONQUIST, 1981). The literature refers frequently to this species as *Myrica faya* Aiton and only after 2002, the change of genus made by WILBUR in 1994 starts to be recognized (STAPLES *et al.*, 2002). HERBET (2005) publishes a dichotomous key that distinguishes the genus *Morella* from the genus *Myrica*. According to this key the plants of the genus *Morella* distinguish from that ones of the genus *Myrica*, because they are evergreen shrubs or trees, with not sunken stomata, inflorescences borne on the present year's growth, and papillose fleshy fruits bird-dispersed. *M. faya* is found in the Azorean Subprovince of the European Atlantic Province (RIVAS-MARTINEZ, 2002) and in the essentially coastal Gaditano-Onubo-Algarviense Biogeographic Province, which stretches out from the River branch of Aveiro to the beaches of Costa del Sol and to the sandstone Gaditano Mountains of Gibraltar Field (COSTA *et al.*, 1998). *M. faya* is considered to be a native species of the Azorean islands (SILVA *et al.*, 2010), Madeira Island (JARDIM and SEQUEIRA, 2008), Canaries (BRAMWELL and BRAMWELL, 1990) e Iberian Peninsula (SCHAEFER, 2005; AGUIAR and PINTO, 2007). This species was introduced in Florida (HODGES and GARDNER, 1985), New Zealand (OWEN, 1997) and Australia (CSURHES and EDWARDS, 1998). In Hawaii, where it was introduced by Portuguese immigrants at the end of the 19th century (FOSBERG, 1937; KIM, 1969), is now considered an invasive species (SMITH, 1985; SCHAEFER, 2005). This dioecious evergreen shrub or tree up to 10 (-16) m displays oblong-cuneate and entire to crenate-serrate leaves (4-11 x 0.8-4 cm), naked flowers in axillary branched catkins borne on the present year's growth, fleshy drupes, formed by 2-5 fused endocarps turning and black in maturation (4-6 mm ∅) (FERNANDES and FERNANDES, 1987; FRANCO, 1971; WALKER, 1990; SCHAEFER, 2005). At the Azores this species is known by the local names of faia (DROUET, 1866), faia-da-terra (PALHINHA, 1966), faia-da-ilhas and samouco (FEIJÃO, 1960; SCHAEFER, the blooming season occurs from March to April (SCHAEFER, 2005), and the ripened fruits can be found between August to October (SILVA and TAVARES, 1997). Endozoocoric seed dispersal by *Turdus merula azorensis* and *Columba palumbas azorica* (DIAS, 1996; SILVA and TAVARES, 1997) and pluvial dispersion are both present. *M. faya* is a characteristic species of the following native Azorean plant communities: coastal 'faia' bushes, 'faia' woods and 'faia' forests (DIAS, 1996).

This species found in all the Azorean islands, usually below 600m, with the exception of Pico Island (up to 1000m of altitude) (SJÖGREN, 1973; FERNANDES and FERNANDES, 1987; SCHAEFER, 2005). The original species distribution on the islands strongly decreased due to human occupation and competition with exotic invasive species as *Pittosporum undulatum* (FERNANDES and FERNANDES, 1987), nevertheless, the *M. faya* ability to fix nitrogen (TURNER and VITOUSEK, 1987), the high seed production and the effectiveness of seed dispersal give to the species some resilience. At the Azores this plant is still planted as shelter hedges for cultures (DROUET, 1866; SJÖGREN, 1984; FERNANDES and FERNANDES, 1987), the wood was used as combustible, for charcoal production, and carpentry, the bark was used in leathers tanning, the cattle can feed on their leaves, and the fruits were used to make compotes (RIBEIRO, 1946; DIAS, 1996; VILELA, 2007). The ingestion of mature fruits and bark decoctions were used on the islands as anti-diarrhea medicine (BOTELHO, 2007).

In the Hawaii, WALKER (1990) studied the effects of fruit age, endocarp scarification and passage through bird guts on *M. faya* germination. This author found that germination of *M. faya* seeds was > 80% at 10 weeks, declining to 30% after 78 weeks of dry storage, mesocarp removal and endocarp scarification slightly increased germination at 15 weeks but not at 92 weeks, and that passage through birds had no effect on the germination percentages. At Azores SILVA and TAVARES (1997) studying *M. faya* endocarps germination at Ponta Delgada weather conditions obtained lower percentages of germination (20%), but they also found that that endocarps passage through *Turdus merula azorensis* guts had no effect on the germination percentages (19%). Also leaf litter from *M. faya* trees reduces germination (WALKER, 1990) and germination is enhanced at intermediate shade BINGGELI (1998). Nowadays, the governmental services at Azores and the 'Sustainable Laurel forest' project are producing this species by seed for landscape conservation and for *Pyrrhula murina* wildlife habitat restoration (LIFE-LAURISILVA, 2012); seeds are placed to germinate in flowerbeds, on soil or *Sphagnum* spp. and after germination seedlings are transferred to local soil; nevertheless no published data was found on seed germination characteristics or seedling development. Regarding vegetative production, propagation of *Morella* spp. during the summer and autumn by semi-hardwood cuttings is mentioned by WASSON (2004) while DEHGAN (1998) refers that *M. cerifera* can be propagated by apical softwood cuttings.

Material and methods

Plant material. Fruits of *M. faya* were harvested on September 2011, on 30 trees of one population located at 'Água-de-pau' (São Miguel Island). The fruits were left to dry at room temperature until the establishment of seed trials.

Pretreatments. Chemical scarification of fruits was made by one hour immersion in sulphuric acid (98%) on an Erlenmeyer placed above ice. Imbibitions were made with distilled water during 96 h, with 3 changes of water. Scarified endocarps were imbibed after scarification. Scarified and imbibed seed were placed on *Shagnum* ssp. and stratified at dark, with 4ºC during 2 or 4 weeks.

Germination trials. Each germination trial consisted in 4 repetitions of 100 seeds placed on *Shagnum* ssp. inside 10 cm diameter propylene translucent Petri dishes. In the germination chambers the following temperatures and photoperiods (for each 24 h) were tested: 1) 15ºC, 8h; 2) 20ºC, 16h; 3) 4 weeks at 15ºC, 8h and after 20ºC,16h; 4) 8 weeks at 15ºC, 8h and after 20ºC, 16h; 5) 12 weeks at 15ºC, 8h and after 20ºC, 16h. In the nursery trials, the open Petri dishes were protected of direct rainfall and in intermediate shade conditions.

Seedling development trials. Seedlings with two cotyledons were planted on: A) blond peat (Tref®) : perlite (2:1) (watered with liquid universal fertilizer), B) BVB (blocking compost for horticulture, Bas Van Burren®, Maasland, the Netherlands) : perlite (2:1) e C) *M. faya* habitat soil (from landslides over roads). The substrates were placed on plastic trays with 40 cells, each one with 35 cm^3 and 5 cm high. Seedlings were watered once a week. In March these trays were placed at nursery protected from direct rainfall and in intermediate shade conditions during 12 weeks.

Cutting trials. Semi hardwood cuttings and softwood cuttings were harvested on October 2008 and April 2012 respectively, on 3 trees of *M. faya* at Ponta Delgada. In the basal end of the 5 to 7 cm cuttings a longitudinal and superficial wound 1cm long was made. These wounds were placed 1 minute in water or in Indole-3-butyric acid (IBA) solution (0.4%) before their plantation. Semi-hardwood cuttings were planted in the 40 cells plastic trays with BVB: perlite (3:1), at 20ºC natural day light. Softwood cuttings were placed in an aeroponic tank (X-strem®) with capacity for 105 cuttings, at 20ºC natural day light.

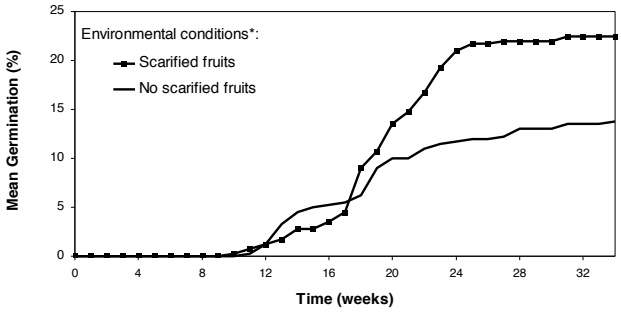
Data analysis. In the germination trials the number of emergent radicles was counted weekly. For each trial the mean number of days to first radicle emergence (latency period), the germination mean time (GMT) and the percentage of germination were calculated. GMT was calculated using the HARRINGTON (1963) formula. For seedlings development trials, the number of surviving plants, the number of leaves per plant, and their heights were calculated after 12 weeks of culture. For data represented as proportions a χ^2 test was used to analyse the contingency tables and when the replicate results were homogeneous, the total χ^2 was used. A t-student test or a single factor analysis of variance (ANOVA) was performed with data represented as measurements or counts; the homogeneity of variances was verified through the Levene statistic and Tukey's test was used for the parametric multiple comparisons of means. When variances homogeneity was not observed, data were statistically compared using the Mann–Whitney U test or Kruskal-Wallis non-parametric test for variance analysis, and Tukey-type non-parametric test for multiple comparisons. In the cutting trials no statistical treatment was performed since 100% mortality was achieved after two months of culture.

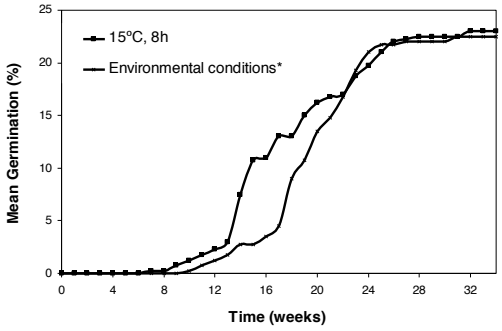
Results

Effect of chemical scarification on germination characteristics. After 34 weeks the chemical scarification of *M. faya* fruits significantly affected the percentages of germination but not the latency period or the GMT (Table 1, Figure 1A).

Table 1 *- Effect of chemical scarification on germination characteristics after 34 weeks (trial initiated on October at Ponta Delgada environmental temperature and photoperiod conditions). GMT, Germination Mean Time. Mean values ± standard deviation. Within columns means separation at 5% level is indicated with different letters.*

Pretreatment: chemical scarification	**N**	**Latency (days)**	**GMT (days)**	**Germination (%)**
Yes	4x100	82,3 ± 12,0 a	133,5 ± 7,1 a	22,5 ± 3,1 a
No	4x100	78,8 ± 6,7 a	120,9 ± 16,8 a	14,0 ± 9,2 b





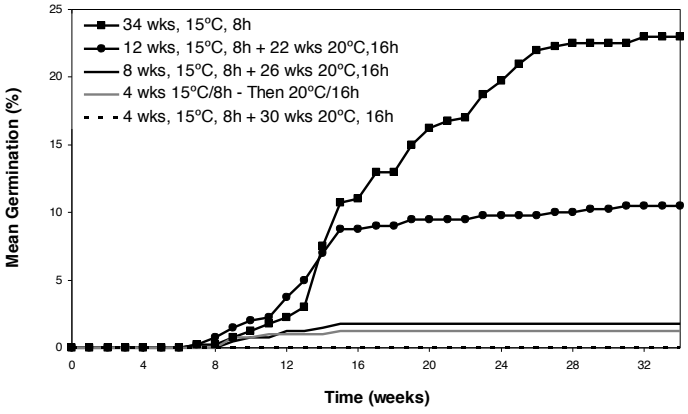


Figure 1 *- Morella faya. A. Cumulative germination curves obtained with scarified and no scarified fruits. B and C. Cumulative germination curves obtained with different regimens of temperature and photoperiod. *Environmental conditions at Ponta Delgada from October to June.*

Effect of temperature and photoperiod regimens on germination characteristics. After 34 weeks the temperature and photoperiod regimens significantly affected the percentages of germination and the GMT but not the latency (Table 2); the best percentages of germination were obtained under a regime of 15ºC and 8h photoperiod or under environmental conditions at Ponta Delgada from October to June (Figure 1B,C).

***Table 2** - Effect of temperature and photoperiod regimens on germination characteristics after 34 weeks (wks). GMT, Germination Mean Time. Mean values ± standard deviation. Within columns means separation at 5% level is indicated with different letters.*

Time span, temperature, photoperiod	N	Latency (days)	TMG (days)	Germination (%)
34 wks, 15ºC, 8h	4x 100	71,8 ± 16,5 a	125,9 ± 6,1 a	23,0 ± 5,5 a
34 wks, 20ºC, 16h	4x 100	-	-	0,0 ±0,0 d
4 wks, 15ºC, 8h + 30 wks 20ºC, 16h	4x 100	70,0 ± 9,9 a	75,3 ± 2,5 b	1,0 ± 2,5 c
8 wks, 15ºC, 8h + 26 wks 20ºC,16h	4x 100	72,3 ± 10,7 a	81,7 ± 4,0 b	2,0 ± 4,0 c
12 wks, 15ºC, 8h + 22 wks 20ºC,16h	4x 100	55,0 ± 5,7 a	92,3 ± 13,3 b	11,0 ± 13,3 b
34 wks, environmental conditions*	4x 100	82,3 ± 12,0 a	133,5 ± 7,1 a	22,5 ± 3,1 a

**trial initiated on October at Ponta Delgada*

Effect of stratification on germination characteristics. From Table 3 we verified that after 34 weeks of culture stratification did not manage to substitute the regimen of 15ºC and 8h photoperiod in endocarps' germination.

***Table 3** - Effect of stratification (4ºC, dark) on germination characteristics after 34 weeks. GMT, Germination Mean Time. Mean values ± standard deviation. Within columns means separation at 5% level is indicated with different letters.*

Stratification (weeks)	Temperature / photoperiod	N	Latency (days)	GMT (days)	Germination (%)
0	15ºC - 8h	4x100	71,8 ± 16,5 a	125,9 ± 6,1 a	23,0 ± 5,5 a
0	20ºC - 16h	4x100	-	-	0,0 ±0,0 b
2	20ºC - 16h	4x100	105,0 a	105,0 b	0 ± 0,50 b
4	20ºC - 16h	4x100	105,0 a	105,0 b	0 ± 0,50 b

Effect of substrate type on survival and development of seedlings. M. faya habitat soil significantly enhanced the survival percentage of seedlings, nevertheless the number of leaves per plant and the plants' height was significantly superior in the mixture BVB : P (2 : 1) (Table IV).

***Table 4** - Effect of stratification (4ºC, dark) on germination characteristics after 34 weeks. GMT, Germination Mean Time. Mean values ± standard deviation. Within columns means separation at 5% level is indicated with different letters.*

Substrate	N	Survival (%)	Leaves (nº)	Height (cm)
Soil from *M.faya* habitat	2x40	86,3 ± 8,8 a	8,4 ± 1,2 a	2,3 ± 0,6 a
BVB:Perlite (2:1)	2x40	58,8 ± 2,3 b	10,6 ± 2,6 b	3,0 ± 0,8 b
Peat:Perlite (2:1)	2x40	0,0 ± 0,0 c	-	-

Discussion

Effect of chemical scarification on germination characteristics. It has been proposed that fruits' ingestion by birds favours germination trough the pulp removal (MAYER and POLJAKOFF-MAYBER, 1989) and/or trough structure modification of seminal involucres (AGAMI and WAISEL, 1986). It is accepted that grinding and abrasion during ingestion enhances the permeability of seminal involucres to water and oxygen, favouring germination (BARNEA *et al.*, 1991). Considering the *M. faya* endozoocoric seed dispersal of WALKER (1990) and SILVA and TAVARES (1997), did not found any evidence that acid treatment during gut passage modifies the characteristics of germination. In this work, we start from the principle the submission to acidic conditions alters the involucres permeability and germination (SCHUPP *et al.*, 2010), not to test the importance of seed ingestion by birds on seed germination, but to decrease the time for seedlings production by seed (ISTA, 2005). Our results show that in the first 6 months (168 days), germination percentage is enhanced by endocarps immersion in sulphuric acid (98%. 1 h), but after 8.5 months seeds continued to germinate erratically, which agrees with WALKER (1990) data, nevertheless here the effect was only visible after 140 days and not after 15 days.

Effect of temperature and photoperiod regimens on germination characteristics. At the Azores the hastening period of fruit maturation occurs between September and October. The mature fruits can be ingested by birds; if not the strong winds and rains contribute to pull out the fruits and in contact with soil the pulp of rain washed fruits is eaten or simply rot. During the winter the endocarps are released from their pulps and maintained in humid conditions. The mean temperature in February on the distribution area of this species is not superior to 15ºC (FORJAZ, 2004; COSTA *et al.*, 2012; PORTAL DA BIODIVERSIDADE DOS AÇORES, 2012) while in August the mean temperature is superior to 20ºC in the majority of the distribution area. Our results obtained in germination chambers illustrate that even that the seeds are freed of the pulp and that the endocarps' permeability is improved through his scarification, the seed needs temperatures inferior to 20ºC to germinate. The results suggest that mobilization of viable seeds preserved in a germplasm bank should be done during October in environmental conditions; while the mobilization of these seeds out of this time should resort to germination chambers with a temperature of 15ºC (and 8h photoperiod). Also, the submission of the seeds to shorter periods of 15ºC always turned in the reduction of the percentages of germination, not constituting therefore alternative to the production of the plants under a continuous regime of 15ºC or in environmental conditions in October. Regarding to the light effect on germination, WALKER (1990) in Hawaii describes the increase of germination percentages in a regime of intermediate shadow, ARÉVALO and FERNÁNDEZ-PALACIOS (2003) report that in Tenerife *M. faya* seeds reveal positive photosensitivity, classifying this species as shadow intolerant. In our study the use of *Sphagum* spp. as germination substrate allowed a situation of partial shadow. Since we have not found any information about the effect of photoperiod duration on *M. faya* germination, we choose to associate the short photoperiod (8 h) to the lowest temperature (15ºC) close to winter days and the longest photoperiod (16 h) to the highest temperature (20ºC) close to summer days.

Effect of stratification on germination characteristics. In spite of lower temperatures were necessary to the germination of this species, the vernalization by submission to 2 periods of stratification at 4ºC (ISTA, 2005) obstructed the germination during the germination test. These results suggest that the temperature appropriate to the germination: 15ºC, cannot be substituted for a short period of time at inferior temperatures. The failure of stratification might be explained by the reason that 4ºC and near temperatures are not registered in the area of distribution of this

species. The endocarps submission to temperatures far from the least ones to take place germination can result in the development of secondary dormancy (TAIZ and ZEIGER, 2006).

Effect of substrate type on survival and development of seedlings. The higher percentage of seedlings survival in the substrate of their natural habitat can be generically explained by the species' adaptation to the physical (e.g. texture), chemical (e.g. pH) and biological (e.g. presence of symbionts) characteristics of this substrate. However in volcanic grounds the nitrogen contents limits the plants' vegetative growth (TURNER and VITOUSEK, 1987). In spite of *M. faya* thrive in soils nitrogen poor, forming symbiotic associations with *Frankia* spp. (MIGUEL and RODRIGUES-BARRUECO, 1974; MIAN *et al*., 1976), at the moment of the plantation, the radicular system of the seedlings still do not present any nodulation. Therefore, the presence of nitrogen, phosphorous and potassium in the mixture BVB:Perlite, allowed a quicker development in the space of 12 weeks regarding the natural substrate. Finally, after the failure obtained in the mixture peat: perlite, the pH was measured and resulted too low (pH 4) which might explain the quick (3 days) and uniform death answer of the seedlings. The pH of the substrate BVB (between 5.6 and 5.8) is closer to the pH andosols of the Azores (DAVIES, 1962; SILVA, 2001).

Cuttings. In the conditions here tested, the use of the two types of cuttings indicated in the references for the genus *Morella* (DEHGAN, 1998; WASSON, 2004), did not allow the establishment of *M. faya* by cuttings, suggesting that this may be a difficult species to propagate by cuttings.

Conclusion. In spite of the modest percentages of germination after 8.5 months, the great abundance of seeds, the vigour of seedlings growth, and the resilience of this species regarding the exotica species that compete for the same habitat in the Azores, substantiate the propagation of this species by seed, not justifying at this moment the investment to carry out cutting trials.

Acknowledgements

Thanks to Geotrota Lda (*www.geotrota.pt*), the Azorean Center of Research and Agrarian Technologies and the University of the Azores by financial support.

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