Silva Lusitana, **25**(1): 51- 77, 2017
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Propagation of Nine Endemic Plant Species from Madeira Island (Portugal)

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Abstract. Efficient propagation of endangered plant species is a critical factor in successful ecological restoration and conscientious habitat management. Hence, propagation trials of nine endemic plant species of Madeira (*Anthyllis lemanniana* Lowe, *Armeria maderensis* Lowe, *Cedronella canariensis* (L.) Webb & Berthel., *Erica maderensis* (Benth.) Bornm., *Genista tenera* (Jacq. ex Murray) Kuntze, *Helichrysum melaleucum* Rchb. ex Holl, *Pericallis aurita* (L'Her.) B. Nord., *Sideritis candicans* Aiton and *Teline maderensis* Webb & Berthel.) were carried out. Plant propagation requirements and their sexual and vegetative propagation methods were studied. Seed germination success varied between species. Germination rate exceeded 70% in six out of nine species, being lower than 30% in *Pericallis aurita*, while *H. melaleucum* seeds did not germinate. Vegetative propagation yielded lower success rates, with three species (*Erica maderensis*, *Genista tenera* and *Teline maderensis*) unable to establish roots, and three species (*Helichrysum melaleucum*, *Pericallis aurita* and *Sideritis candicans*) exceeding 60% of the rooting success.

Establishment of the propagation requirements of these species could be regarded an important tool for supporting Madeira's flora conservation programs.

Key words: Seed propagation, vegetative propagation, plant conservation

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Propagação de nove espécies de plantas endémicas da ilha da Madeira (Portugal)
Sumário. A propagação eficaz de espécies de plantas ameaçadas pode ser um fator crítico numa restauração ecológica de sucesso e numa gestão consciente de habitats.
Por conseguinte, foram realizadas ensaios de propagação de nove espécies de plantas endémicas da Madeira (*Anthyllis lemanniana* Lowe, *Armeria maderensis* Lowe, *Cedronella canariensis* (L.) Webb & Berthel., *Erica maderensis* (Benth.) Bornm., *Genista tenera* (Jacq. Ex Murray) Kuntze, *Helichrysum melaleucum* Rchb. Ex Holl, *Pericallis aurita* (L'Her.) B. Nord., *Sideritis candicans* Aiton e *Teline maderensis* Webb & Berthel.).

Foram analisados os requisitos de propagação destas espécies através de métodos de propagação sexual e vegetativa.

O sucesso da germinação das sementes variou entre as espécies. A taxa de germinação foi superior a 70% em seis das nove espécies, sendo inferior a 30% em *Pericallis aurita*, e de 0% para *H. melaleucum*. A propagação vegetativa apresentou taxas de sucesso inferiores, com três espécies (*Erica maderensis*, *Genista tenera* e *Teline maderensis*) incapazes de estabelecer raízes. Por outro lado, três espécies apresentaram taxas de enraizamento superiores a 60% (*Helichrysum melaleucum*, *Pericallis aurita* e *Sideritis candicans*).

O estabelecimento dos requisitos de propagação dessas espécies pode ser considerado como uma ferramenta importante na conservação da flora da Madeira.
Palavras-chave: Propagação de sementes, propagação vegetativa, conservação de plantas

Propagation des neuf espèces de plantes endémiques de l'île de Madère (Portugal)
Résumé. La propagation efficace des espèces végétales menacées est un facteur critique pour la restauration écologique réussie et la gestion consciencieuse de l'habitat.

Ainsi, ont été réalisés les essais de propagation de neuf espèces de plantes endémiques de Madère (*Anthyllis lemanniana* Lowe, *Armeria maderensis* Lowe, *Cedronella canariensis* (L.) Webb et Berthel., *Erica maderensis* (Benth.) Bornm., *Genista tenera* (Jacq. Ex Murray) Kuntze, *Helichrysum melaleucum* Rchb. Ex Holl, *Pericallis aurita* (L'Her.) B. Nord., *Sideritis candicans* Aiton et *Teline maderensis* Webb & Berthel.).

Les besoins de propagation des plantes et leurs méthodes de propagation sexuelle et végétative ont été étudiés.

Le succès de la germination des semences variait d'une espèce à l'autre. Le taux de germination a dépassé 70% chez six espèces sur neuf, soit moins de 30% chez *Pericallis aurita*, alors que les graines de *H. melaleucum* n'ont pas germé.

La multiplication végétative a donné des résultats inférieurs avec trois espèces (*Erica maderensis*, *Genista tenera* et *Teline maderensis*) incapables d'établir des racines, alors que trois espèces (*Helichrysum melaleucum*, *Pericallis aurita* et *Sideritis candicans*) excédant 60% du succès d'enracinement.

L'établissement des besoins de propagation de ces espèces pourrait être considéré comme un outil important pour soutenir les programmes de conservation de la flore de Madère.
Mots-clés: Propagation de semences, multiplication végétative, conservation des plantes

Introduction

Oceanic islands tend to have unique floras, with higher number of endemisms, with the Archipelagos of Madeira and Selvagens being a prime example. Their flora comprises of 1,204 vascular plant species, 780 native and 154 endemics, with endemism representing 13% of the overall diversity (JARDIM & SEQUEIRA, 2008). Madeira Island has the second richest flora of Macaronesia, harboring the highest biodiversity *per* area (BORGES *et al*., 2008).

Undoubtedly, Madeira is included in one of Europe's hotspots of biodiversity, being integrated in the biodiversity hotspot of the Mediterranean (MYERS *et al*., 2000; BORGES *et al*., 2008), and a recognized site for conservation priorities (BORGES *et al*., 2008; BILZ *et al*., 2011). Many plant species have small population sizes or are threatened and, therefore, there is a need for *ex situ* and *in situ* conservation efforts in order to safeguard this unique natural heritage.

The effects of climate changes and anthropogenic activities on native ecosystems are also affecting Madeira. Over the last years we have noticed some vulnerability of Madeira's plant cover concerning forest fires and floods. Historical climatic records have shown a progressive climate warming throughout the last century, reaching up to 0.51°C per decade in some areas, and it is expected to increase in the decades to follow (MIRANDA *et al*., 2006; SAUTER *et al*., 2013). A considerable reduction in annual precipitation (up to 30%) (MIRANDA *et al*., 2006; CRUZ *et al*., 2009; SAUTER *et al*., 2013) and water availability (up to 40-50%) (OLIVEIRA *et al*., 2006) are also predicted. These constrains will have a critical impact on water resources of small creeks and ground water aquifers, threatening natural ecosystems (SAUTER *et al*., 2013). The expected changes will lead to the expansion of invasive species, an altitudinal shift in the native vegetation that will undergo a decrease in its suitable area (FREITAS & COUTINHO, 2006; CRUZ *et al*., 2009), and an expected increase of plant disease and pests (CORREIA *et al*., 2006).

Consequently, under these conditions, mountain ecosystems and their vegetation will suffer an increasing pressure. The actions to restore these fragile ecosystems will be of great importance to reverse the trend of species loss (DORNER, 2002). The use of native species is advantageous to successful ecosystem restoration. Native plants form self-sustaining, locally adapted communities that do not require great maintenance efforts, and hence tend to resist damage from numerous external stress factors, such as fires, drought and diseases (DORNER, 2002).

The nine plant species studied in this work (*Anthyllis lemanniana* Lowe, *Armeria maderensis* Lowe, *Cedronella canariensis* (L.) Webb & Berthel., *Erica maderensis* (Benth.) Bornm., *Genista tenera* (Jacq. ex Murray) Kuntze, *Helichrysum melaleucum* Rchb. ex Holl, *Pericallis aurita* (L'Her.) B. Nord., *Sideritis candicans* Aiton and *Teline maderensis* Webb & Berthel.) are prime examples of native plant species that should be used in the ecological restoration of these ecosystems as they represent a succession stage that leads to the climax stage of the target habitat (CAPELO *et al*., 2004). They represent the understory and edges of the Laurel and Heaths forests (CAPELO *et al*., 2004), and are expected to be successful colonizers after a habitat disturbance.

Although seed propagation is a preferred method for habitat restoration or plant recovery purposes due to genetic diversity conservation (BONNER & KARRFALT, 2008), low seed availability favors vegetative propagation as a faster alternative and an efficient method for the multiplication of some species.

Furthermore, the seeds of many native wild species require specific conditions to germinate (MEYER, 2006), resulting from their adaptation to ecological and environmental cues that trigger germination (LUNA *et al*., 2009). This is due to seed dormancy mechanisms developed in nature to increase the chances of seedling survival by preventing germination during unsuitable ecological conditions or by delaying germination (MEYER, 2006; BONNER & KARRFALT, 2008; TAIZ & ZEIGER, 2010).

A variety of treatments can be used to break dormancy, including scarification (mechanical or chemical), soaking, light, germination stimulators (such as gibberellins, GA) and stratification (or chilling) (BONNER & KARRFALT, 2008; LUNA *et al*., 2009; HARTMANN *et al*., 2011).

When seed germination is unsuccessful, vegetative propagation by rooted cuttings, air layering, grafting, budding or micropropagation could be attempted as alternative methods of plant propagation (BONNER & KARRFALT, 2008). Some plant species, including those in wild habitats, develop vegetative reproduction as an alternative way of reproduction. According to the best of our knowledge, information about seed germination success and vegetative propagation of these nine species has not been published to date.

The main objective of this study was to attain the know-how on the propagation requirements of the nine target plant species and establish propagation protocols considering both the seed and vegetative methods. Furthermore, the study of their propagation may assist in the conservation of these *taxa,* from which three species face the risk of extinction (or are vulnerable) and have low population counts (*A. lemanniana, A. maderensis* and *S. candicans*)

(JARDIM *et al.*, 2006), and two of which are on the list of the 100 taxa for priority management in Madeira (*A. lemanniana, A. maderensis*) (FARIA *et al.*, 2008). Additionally, *A. lemanniana* is listed in the Bern Convention and Habitats Directive (Annexes II, IV) (JARDIM & SEQUEIRA, 2008; BILZ *et al.*, 2011). Moreover, the species studied in this work are part of protected habitats, namely the "Endemic Macaronesian Heaths" and "Macaronesian laurel forests (*Laurus, Ocotea*)", both priority habitats (EUROPEAN COMMISSION, 2013).

Materials and Methods

Plant material

The reproductive features of nine species belonging to five families were studied: *A. lemanniana*, *G. tenera* and *T. maderensis* from Fabaceae; *C. canariensis* and *S. candicans* (Lamiaceae); *H. melaleucum* and *P. aurita* (Asteraceae); *A. maderensis* (Plumbaginaceae); and *E. maderensis* (Ericaceae) (Table 1).

Plant material was collected from 18 wild populations, located along different altitudinal ranges. On the south face of the island, the collection sites were Assomada (*c.* 200 m a.s.l.), the Ecological Park of Funchal (*c.* 470 m a.s.l.), Monte da Tabaiba (*c.* 272 m a.s.l), near Poiso (*c.* 1,414 m a.s.l), Encumeada (800 – 900 m a.s.l.), Levada da Serra do Faial (750 – 800 m a.s.l.) and Ribeiro Frio (830 – 860 m a.s.l). On the north face, the surveyed sites included Folhadal (1,000 m a.s.l.), Funduras (*c.* 597 m a.s.l), Portela (*c.* 626 m a.s.l), Achadas da Cruz (575 m a.s.l.), Levada da Ribeira da Janela (430 – 460 m a.s.l.), Chão da Ribeira (*c.* 500 m a.s.l.), Levada dos Cedros (840 – 1,130 m a.s.l), Lombo do Mouro (*c.* 1,280 m a.s.l). Additionally, several sites cross through the island, encompassing both the north and south faces, including the Central Mountain Massif, namely Caminho Real da Encumeada (*c.* 940 m a.s.l.), Paúl da Serra (*c.* 1,500 m a.s.l) and the Vereda do Arieiro (1,542 – 1,818 m a.s.l).

In general, plants grown at the collection sites bloom and set fruits from spring through autumn, depending, however, on climatic conditions (PRESS & SHORT, 1994; JARDIM & FRANCISCO, 2000). In most cases, vegetative plant material was collected between May and November 2011 and seeds were collected from June through November 2011.

Table 1 – Plants species studied, with description of their biological type, ecology and collection sites (PRESS & SHORT, 1994; JARDIM *et al.*, 2007)

Species (Family)	**Life Form Life cycle type**	**Ecology**	**Altitudinal range [m] a.s.l.**	**Site and Date of Seed collection**
• *Anthyllis lemanniana* (Fabaceae)	Decumbent to ascending, much–branched from base, herbaceous woody–based perennial. Polycarpic.	Cliffs, steep rocks and ledges in the mountainous central region of Madeira Island.	1,200 – 1,800 m	Pico do Arieiro, 08/2011
• *Armeria maderensis* (Plumbaginaceae)	Tufted herb with woody, branched stock. Monocarpic.	Exposed rocky and sandy sites on the summits of the high central peaks of Madeira Island.	Highest altitudes (*ca.* 1,800 m)	Pico do Arieiro, 07/2011, 08/2011
○*Cedronella canariensis* (Lamiaceae)	Perennial herb to small bush, woody at base. Polycarpic.	Laurisilva and shady sites of Madeira Island.	Above 500 m	Levada dos Cedros, 07/2011, 09/2011; Ribeiro Frio, 07/2011, 08/2011; Poiso, 07/2011; Ecological Park of Funchal, 07/2011; Lombo do Mouro; 08/2011; Encumeada, 08/2011, 09/2011
• *Erica maderensis* (Ericaceae)	Stocky shrub up to 80 cm, usually prostrate. Woody perennial. Polycarpic.	In heath sites and on bare rock faces in the mountainous central region of Madeira Island.	1,400 – 1,800 m	Only vegetative propagation
• *Genista tenera* (Jacq. ex Murray) Kuntze (Fabaceae)	Unarmed woody perennial shrub, up to 2,5 m. Polycarpic.	Exposed rocky cliffs and ravines, mainly on the south side.	From sea level up to 1,700 m	Caminho Real da Encumeada, 07/2011; Monte da Tabaiba, 07/2011; Assomada; 07/2011;
• *Helichrysum melaleucum* Rchb. ex Holl (Asteraceae)	Woody–based small shrub, perennial, up to 100 cm. Polycarpic.	Exposed rocky cliffs and steep rocks	From sea level up to 1,700 m	Achadas da Cruz, 08/2011
• *Pericallis aurita* (L'Her.) B. Nord. (Asteraceae)	Slender, open semi–hardwood perennial shrub up to 1.5 m. Polycarpic.	Laurisilva and ravines and on rocky slopes in the higher parts in the interior of Madeira Island.	Altitudes above 1,000 m.	Caminho Real da Encumeada, 07/2011; Encumeada, 07/2011; Funduras, 08/2011; Portela, 08/2011; Paúl da Serra, 08/2011; Achadas da Cruz, 08/2011
• *Sideritis candicans* Aiton (Lamiaceae)	Herbaceous small shrub, perennial, 45 – 100 cm. Polycarpic.	Laurisilva and clearings and open, sunny sites in Madeira Island.	600 – 1,700 m	Lombo do Mouro, 08/2011; Encumeada, 08/2011
• *Teline maderensis* Webb & Berthel. (Fabaceae)	Unarmed woody perennial evergreen shrubs or small tree, up to 6 m. Polycarpic.	Laurisilva and rocky wooded ravines, and maritime cliffs.	From sea level up to the highest altitudes	Encumeada, 08/2011; Levada da Ribeira da Janela, 08/2011; Ribeiro Frio, 11/2011

• Endemic to Madeira; ○Endemic to Macaronesia.

The seeds were air–dried in trays for a few days, and subsequently manually cleaned. Any visually malformed or immature seeds were rejected. The seeds were then stored in dry conditions at room temperature in glass containers with silica–gel. The vegetative material was propagated, at the most, a day after collection.

The study was conducted in a non-acclimatized greenhouse located at the Floriculture Centre (Lugar de Baixo, Ponta do Sol) on the southern coast of Madeira, 10 – 40 m a.s.l. The climate is dry infra-mediterranean, characterized by a long dry season during the summer months (MESQUITA *et al*., 2004). The greenhouse has a roof covered with hard plastic panels, and open walls covered with nets. The temperatures inside the structure ranged from 29°C (day) to 19°C (night) during summer/autumn, while in winter temperatures varied between 23-24°C (day) and 14-15°C (night).

Seven seed pre-treatments and three vegetative propagation methods were used to study their effects on seed germination and plant propagation.

Final germination percentage was calculated for seed propagation and the rooting percentage was reported for the vegetative propagation.

Statistical analysis for seed germination and vegetative propagation data analysis was performed using the SPSS Statistics 22 software package. Both parametric and nonparametric tests were performed, and the p-value (<0.05) was calculated to establish significant differences between each treatment. For statistical analysis, data as percentages were transformed to arcsine$\sqrt{}$ $(x/100)$.

Seed propagation trials

Germination trials were carried out between June 2011 and April 2012. In general, seven seed treatments were tested, namely: a) control, without treatment; b) mechanical scarification; c) cold water immersion; d) mechanical scarification, followed by immersion in water; e) gibberellic acid (GA_3) treatment; f) scarification in hot water; and g) scarification in boiling water (Table 2).

Due to limited seed availability, the above–mentioned treatments, including control tests, were selectively applied only to the species that were indicated as relevant in several bibliographical references. Specifically, control tests for *C. canariensis* and *T. maderensis* were not performed based on the seed hardness of this species that would turn the seed unable to imbibe water, and therefore, unable to germinate (HARTMANN *et al*., 2011). Similarly, prompted by the bibliographical references, control test were not performed on *A. lemanniana* (DOUSSI & THANOS, 1993, 1994; IBAÑES & PASSERA, 1997; PRIETO *et al.*, 2004; MORBIDONI *et al.*, 2008). The rarity of some species and scarcity of seed production affected the seed sample size, which was not uniform throughout the tests. Therefore, each trial contained a variable number of seeds according to the species, with 2 to 6 replicates (for more details see Table 2).

Table 2 – Final germination percentage (%) of the experimental tests to study the sexual propagation of the target species

	Treatments								
Species	**Seeds per trial**	**Control**	**Mechanical scarification**	**Cold water immersion**	**Mechanical scarification + water immersion**	**GA_3 treatment**	**Hot water scarification**	**Boiling water scarification**	**Cold Stratification**
Anthyllis lemmaniana	1000*	–	97.8% (December); 39.3% (June)	–	–	–	–	–	–
Armeria maderensis	255 (control); 20-30*	36.9% (December); 41.7% (June)	–	3.6% (10h); 21.1% (12h)	–	–	–	–	98.0%
Cedronella canariensis	50 (*; mechanical scarification); 30 – 500 (*; cold water immersion); 30 (scarification + immersion)	–	57.1%; 46%	5.8% (3h25); 7% (18h); 0% (10h); 100% (102) (12h)	13.3%	–	–	–	3.3%
Genista tenera	450 - 511; 10 (*; hot scarification)	24.2%	–	–	–	–	56.2 – 91.6%	3.1%	–
Helichrysum melaleucum	Seeds were not summed	0%	–	–	–	–	–	–	–
Pericallis aurita	1000 (control); 2000*	5.3%	16.8% (September) 29.5% (December)	23.5% (12h)	–	–	–	–	–
Sideritis candicans	40 (*; control); 20 – 30*	72.5% (light); 20% (shade); 36.4-70%; 46.7% (January)	12.5%	25% (10h); 17.2% (24h)	–	25% (T_{amb}); 70% (3°C)	–	–	–
Teline maderensis	20*	–	–	–	40 - 50% (1h30)	–	73.3% (5 min, August); 55 – 85% (5 min, November); 36.4% (10 min)	–	–

If no treatment is mentioned, seed number is applied in all seed treatments. * Indicates trials with 2 to 6 duplicates.

The following are details of seeds pre-treatment:

a. Mechanical scarification – the seeds were rubbed between two sheets of fine–grained sandpaper;
b. Cold-water treatment – the seeds were immersed for different periods, ranging from 30 minutes up to 24 hours, depending on the species;
c. Mechanical scarification plus water treatment – combination of sandpaper scarification, followed by water immersion for 90 min up to 6 h;
d. GA_3 treatment – the seeds were immersed in a 100 ppm solution of GA_3 for 72 h, either at room or cold temperatures (3°C);
e. Scarification with hot water – boiling water was poured for 5 or 10 min on the seeds placed in a glass container;
f. Scarification with boiling water – the seeds were placed in a small strainer and immersed in boiling water (100°C) for 1 min.

An additional treatment was applied to *A. maderensis* and *C. canariensis*, consisting of the seed cold stratification at a temperature between 1 – 6ºC, for 3 months and 1 month respectively, by placing the seeds on a layer of filter paper soaked in distilled water in a sterile Petri dish.

After the treatment, the seeds were sown in germination trays filled with a substrate mixture of soil, peat and perlite (2:1:1), which was used in the majority of the germination trials. For *A. maderensis* and *S. candicans* a different substrate composition was also tested that included the addition of gravel to the substrate (soil, gravel, peat and perlite, with a ratio of 2:2:1:1).

All the seeds were thoroughly cleaned except for *A. maderensis*, whose seeds were tested with and without their papery bracts.

The germination trays were covered with a glass sheet to maintain humidity and were watered every two days.

The seeds were considered germinated with the radicle emergence through the seed coat (TAIZ & ZEIGER, 2010).

Each trial had duration of six months for *A. lemanniana*, *C. canariensis* and *H. melaleucum*, and ten months for *A. maderensis*, *G. tenera*, *P. aurita*, *S. candicans* and *T. maderensis*.

The number of days to germination of 50% of all germinated seeds (T_{50}) was calculated according to the following formula of BACCHETTA *et al*. (2008):

$$T_{50} = [(N/2 - N_1)\ (T_2 - T_1)]\ + T_1\ /\ N_2 - N_1$$

where, N is the final number of emergence and N_1 and N_2 the cumulative number of seeds germinated by adjacent counts at times T_1 and T_2 respectively, when $N_1 < N/2 < N_2$. The T_{50} values were not calculated when germination was below 5%.

Vegetative propagation trials

Vegetative propagation of *C. canariensis*, *E. maderensis*, *G. tenera*, *H. melaleucum*, *P. aurita*, *S. candicans* and *T. maderensis*, were carried out between June 2011 and April 2012. *A. lemanniana* and *A. maderensis* were not considered suitable for vegetative propagation due to their characteristics, that involve little or no vegetative spread and their highly herbaceous nature.

Simple cuttings were tested for all of the six species in the vegetative propagation trials. Additional approaches included trench layering for *C. canariensis* and *P. aurita*, and heel cuttings for *T. maderensis*. The number of cuttings *per* test was not standardized due to the scarcity of some species in nature, and therefore, their collection in nature was made considering the number of individuals available in the wild populations in a sustainable approach.

To prepare stem cuttings, a slant angle cut was made just below a node and the leaf area was reduced, leaving only 2 – 3 apical leaves. For cuttings that were taken from the middle portion of a branch, a straight cut was made in the apical portion to reduce water loss. Layering was prepared by wounding the nodes. The cuttings were tested with indole-3-butyric acid (IBA) or potassium salt of indole-3-butyric acid (KIBA) and without the rooting hormones. A powder formulation of IBA with different concentrations of IBA was used according to the type of cutting, namely 0.1% w/w IBA for softwood cuttings (IBA 0.1%), 0.3% w/w IBA for semi–hardwood cuttings (IBA 0.3%), and 0.8% w/w IBA for hardwood cuttings (IBA 0.8%).

Cuttings were established in a substrate mixture of soil, peat and perlite (2:1:1). All cuttings were bed in substrate mixture, either in small plastic bags or in styrofoam boxes covered with a glass sheet in order to maintain humidity until the establishment of rooting. The cuttings were watered every two days.

Cedronella canariensis and *P. aurita* were tested for two methods of vegetative propagation: simple stem cuttings (softwood) with a slant angle, as well as trench layering. For the stem cuttings the difference between the absence of hormones and IBA 0.1% was tested. For the trench layering trial, no hormones were used. For the *C. canariensis* tests, 35 cuttings were used for the IBA 0.1% trials and 421 cuttings for the simple cuttings without the hormones. Forty-three cuttings were tested using the layering method. In *P. aurita*, simple cuttings without hormones were used on 17 cuttings, while IBA 0.1% was applied on 46 cuttings. For the layering method, 10 cuttings were tested for each trial (with and without hormones).

Erica maderensis cuttings (hardwood) were tested either without hormones or with IBA 0.8%. For this species each trial consisted of 20 cuttings.

The heel cutting method was applied to *G. tenera*, both softwood and hardwood cuttings, both without hormones, and with IBA 0.1% and 0.8%, respectively. For this species each trial was performed with 25 to 40 cuttings.

For *H. melaleucum*, 1 cm of the epidermal layer from the lower portion of the semi–hardwood cutting was removed in order to increase rooting success (DRAGOVIC, 2009) and dipped in KIBA (500 ppm) or IBA (1,000 ppm), both for 5 seconds. For this species each trial consisted of 20 to 30 cuttings.

For *S. candicans*, semi–hardwood stem cuttings were tested with a quick–dip in IBA (1000 ppm) for periods of 5, 7 and 10 seconds and KIBA (500 ppm) for 7 seconds. For this species, the trial using IBA was performed with 20 cuttings and those using KIBA consisted of seven to nine cuttings.

Teline maderensis was tested for two approaches of the vegetative propagation: simple stem cuttings with a slant angle as well as the heel cutting. For the semi–hardwood stem cuttings the difference between no hormones and a quick–dip in IBA (1,000 ppm) for 10 seconds was tested. For the heel cuttings, IBA 0.1%, 0.3% and 0.8% were used, respectively for softwood, semi–hardwood and hardwood cuttings. For this species each trial was performed on 20 to 43 cuttings.

Results

Seed propagation

The results of seed propagation trials are summarized in Table 2.

Seeds of *Anthyllis lemanniana* were submitted to only one treatment based on the literature data and therefore the control test was not performed. Mechanical scarification was applied in two distinct seed trials (one in June and one in December). We observed that the chosen propagation method was more effective in December, with a success rate of 97.8% (Table 2).

On the other hand, T_{50} was lower in June (5.65 days) by comparison with December (11 days).

Armeria maderensis seeds were tested using three treatments, namely control, cold-water immersion and cold stratification. We found that the highest success rate was reached in seed trials with cold stratification (98.0%, Table 2). The second highest success rate was achieved without any seed treatment, i.e., control (41.7% in June, and 36.9% in December). This species T_{50} showed a high range, fluctuating between 12,65 (control, December) and 63.08 days (control, June); and cold-water immersion yielded a T_{50} of 71.66 days. Regarding trials with cold stratification, T_{50} values were not calculated since all the seeds germinated at approximately the same time, and therefore the formula was not applicable.

Cedronella canariensis seeds were subjected to five treatments (Table 2). Control tests were not performed based on the seed hardness that would make the seed unable to imbibe water, and therefore, unable to germinate. In these cases, the seed usually requires some other kind of treatment to germinate. The most successful was water immersion for 12 h resulting in 100% germination. Mechanical scarification appeared to be the second best method, yielding 57.1% germination. The remaining treatments resulted in a lower germination success, between 0% and 46.0%.

C. canariensis T_{50} values were high. The mechanical scarification treatment attained 90.34 days, while water immersion T_{50} values ranged between 84.25 days (18h), 89.27 days (3h 25 min) and 102 days (12h). Regarding the treatment that combined mechanical scarification and water immersion, all the seeds germinated at approximately the same time, and therefore the formula was not valid and the T_{50} value was not calculated.

Genista tenera germination was tested under three seed treatments, namely, control, immersion in hot water 5 min, and immersion in boiling water 1 min. The most successful was hot water scarification for 5 min, leading to germination between 56.2% and 91.6% (Table 2). The lowest success rate was achieved with the boiling water scarification for 1 min (3.1%). This species trials showed low T_{50} values, namely 13.66 days for the control treatment, and from 13.53 up to 27.08 days for the hot water scarification treatment.

Helichrysum melaleucum tests were deemed unsuccessful with no germination established.

Pericallis aurita seeds were exposed to three seed treatments (Table 2). The best result was attained with the mechanical scarification with a low success rate of 29.5% in December, whereas in September this value was even lower, reaching 16.8% germination. Water immersion yielded 23.5% (August), and control treatment achieved only 5.3%.

P. aurita T_{50} values were similar amongst treatments, with values between 16,16 (December) and 30.89 days (September) with mechanical scarification, with these values increasing with the decrease of germination success. The water immersion trials showed a T_{50} of 28.5 days.

Sideritis candicans seeds were subjected to four treatments, namely control, mechanical scarification, water immersion, and GA_3 dipping either combined with cold stratification at 3°C or sown at room temperature (Table 2). A test was made to verify the best period of the year for the propagation of this species. The highest success rate was achieved in the control trial in August (72.5%), and the tests performed in early January showed 70% germination. On the other hand, control trials performed in September showed lower germination success, between 38.36 and 40%. A test was performed (August) in order to explore the effect of light without seed treatment. We found that light led to a higher germination (72.5%) than the shade conditions (20.0%, Table 2). Another treatment that showed high germination success was GA_3 dipping combined with cold stratification with a 70.0% germination success.

This species yielded different T_{50} values depending on the treatment applied to the seeds. The lowest T_{50} values were found in the seeds treated with GA_3, namely 11 days at 3°C and 19.5 days at room temperature. In the control trials, the lowest T_{50} were from tests performed in January (15.28 days), whilst trials performed between August and September yielded values between 20 and 76.5 days. Concerning T_{50} values found in the other trials, the mechanical scarification trials were found to have a T_{50} of 43.5 days, whilst water immersion reached 29 days.

Teline maderensis seeds were subjected to two treatments, namely to hot water immersion of different durations, and a combination of warm water immersion and mechanical scarification (Table 2). The test with the best results was immersion in hot water for 5 min, with success rates between 55.0% and 85.0%. On the other hand, hot water immersion for 10 min lowered germination to 36.4%. The combination of warm water immersion and mechanical scarification resulted in 40.0 up to 50.0% germination.

T. maderensis T_{50} lowest values were observed with the 5 min hot water treatment, being the lowermost in August (20 days) followed by November (30.11 days). The 10 min trials yielded a T_{50} value of 38 days, and the combination of warm water immersion and mechanical scarification reached 31.67 days.

Regarding the statistical tests, the results demonstrated that there was no statistical difference between treatments, except for *G. tenera*, with a p-value of 0.025 in the one-way ANOVA test.

Vegetative propagation

The results of plant vegetative propagation are summarized in Table 3.

Cedronella canariensis vegetative propagation was performed between May and November using two methods, the simple stem cuttings and the trench layering (Table 3). IBA 0.1% was applied only in May, since the cuttings using this hormone showed a low success rate (3.0%). Regarding cuttings without hormones, the highest success rate was reached in November (48.2%), followed by August and July, both with 41.0% success. The lowest success rate was achieved in May (33.0%). We found that in most cases the rooting occurred within a month from the commencement of the treatments. The trench layering method had the highest success rate in July (30.8%) followed by June (20%).

Propagation success of *Helichrysum melaleucum* greatly depended on the time of experiment (Table 3). Cuttings made in May had a success rate of only 4.0%. On the other hand, cuttings prepared in August showed a 60.0% success rate. These results were achieved only with a 5 second quick–dip in KIBA (500 ppm) since the cuttings tested with IBA (1000 ppm) did not root at all.

In *Pericallis aurita*, the most successful treatment was trench layering with and without hormones (60% and 80%, respectively) (Table 3). On the other hand, simple cuttings proved to be a less effective method, not reaching, overall, higher than 36.9%.

Sideritis candicans cuttings attained a general yield of 47.8% (Table 3). The highest success rate was achieved in May, with a 5 second quick–dip in 1000 ppm IBA (75.0%), followed by trials made in June (70.0%). Cuttings made in August showed a lower success rate, between 10.0% (10 sec quick–dip in 1000 ppm IBA) and 50.0% (5 sec. quick–dip in 1000 ppm IBA), where only rooting nodules where observed. However, due to this species scarcity in nature, few cuttings were tested.

The lowest success of vegetative propagation was recorded for *G. tenera*, *T. maderensis* and *E. maderensis* (all 0%), with the cuttings dying within a month.

In the statistical tests performed for vegetative propagation, the results demonstrated that there was no statistical difference between treatments.

Table 3 – Rooting percentage (%) of the experimental studies of the vegetative propagation treatments applied to each species

	Treatments							
Species	**Stem cuttings –No treatment**	**Stem cuttings – IBA 1000 ppm**	**Stem cuttings – KIBA 500 ppm**	**Softwood stem cuttings – 0.1% p/p IBA**	**Semi-hardwood stem cuttings - 0.3% p/p IBA**	**Hardwood stem cuttings – 0.8% p/p IBA**	**Trench layering**	**Heel Cutting**
Cedronella canariensis	33.0 – 48.2%	–	–	3.0%	–	–	20.0 – 30.8%	–
Erica maderensis	0%	–	–	–	–	0%	–	–
Genista tenera	0%	–	–	0%	–	0%	–	–
Helichrysum melaleucum	–	0%	7.6% (May); 72% (August)	–	–	–	–	–
Pericallis aurita	29.4%	–	–	36.9%	–	–	60.0% (with hormones); 80.0% (without hormones)	–
Sideritis candicans	–	70.0–75.0% (5 sec dip); 22.2% (7 sec dip); 25.0% (10 sec dip)	42.9% (7 sec dip)	–	–	–	–	–
Teline maderensis	0%	0%	–	0%	0%	0%	–	0%

Discussion

There is very little previously published information regarding propagation features of the studied Madeiran species. These species employed several germination strategies, and in the majority of cases, germinated under a variety of settings achieving the highest success under specific conditions. This is consistent with prior findings reporting relationships between the seed traits, dormancy and habitat among species of close genera (MAYA *et al.*, 1988; DOUSSI & THANOS, 1993, 1994; PRESS & SHORT, 1994; THANOS & DOUSSI, 1995; IBAÑEZ & PASSERA, 1997; HANLEY & FENNER, 1998; HEIMBINDER, 2001; AYANOĞLU *et al.*, 2002; ESTRELLES *et al.*, 2004, 2010; PRIETO *et al.*, 2004; GARCÍA *et al.*, 2005; LOPES *et al.*, 2005; SERRANO-BERNARDO *et al.*, 2007; BONNER *et al.*, 2008; MORBIDONI *et al.*, 2008; CERVELLI, 2009; PAPAFOTIOU & KALANTZIS, 2009; KADIS *et al.*, 2010; UÇAR & TURGUT, 2011), as well as habitat of origin (PRESS & SHORT, 1994; JARDIM *et al.*, 2007).

The germination success ranged from 70.0% (*A. lemmaniana*, *A. maderensis*, *C. canariensis*, *G. tenera*, *S. candicans* and *T. maderensis*) to 0% (*H. melaleucum*), while *P. aurita* showed an intermediate germination success (29.5%).

The vegetative propagation does not appear to be an ultimate reproductive strategy within three species that showed no rooting success, and only four species reaching a rooting success higher than 60%.

Anthyllis lemanniana commenced germinating only after a certain period of time after mechanical scarification. These observations point to the assumption that mechanical scarification mimics this species habitat (rocky cliffs in the highest altitudes of the island) and the need for cold temperatures to trigger germination. Therefore, it is safe to assume that the main factors affecting this species germination are the temperature and breaking seed coat dormancy. This is also supported by previous reports documenting that a major characteristic of the *Anthyllis* genus is seedcoat hardness, hence primary dormancy could be broken by the mechanical scarification (DOUSSI & THANOS, 1993, 1994; IBAÑEZ & PASSERA, 1997; HANLEY & FENNER, 1998; PRIETO *et al.*, 2004; MORBIDONI *et al.*, 2008).

In *Armeria maderensis* dormancy breaking treatments were needed to achieve germination success. Plant seeds exhibited a higher germination rates after cold stratification (98.0% germination success), leading to the break of seeds thermodormancy (secondary dormancy). This fact can be correlated with this species habitat in the highest peaks of the island, up to 1,860 m a.s.l. (PRESS & SHORT, 1994; JARDIM & FRANCISCO, 2000).

Cedronella canariensis seeds showed the highest success rates after water immersion (12h, 100%) and mechanical scarification (57.1%). These results point to the water availability and uptake as key requirements for seeds germination, which is consistent with species habitat, where water is typically available in abundance. Therefore, it can be assumed that this species displays physical dormancy (primary dormancy). In fact, germination tests performed by ENSCOBASE (2011) showed that scarification of the seeds (chipped with scalpel) lead to germination between 46 and 100%, whilst the use of sole GA_3 lead to germination success of only 5 to 26%, proving the need for scarification. On the other hand, *C. canariensis* seems to be easily propagated by simple softwood (herbaceous) stem cuttings without rooting hormones requirement, which can be used as an alternative strategy of plant reproduction.

Erica maderensis vegetative propagation trials were unsuccessful. This could point to an existence of a persistent seed bank sufficient to assure survival of the species, and therefore, the plant did not develop robust vegetative reproduction strategies. Additional experiments involving the treatment with heat or smoke could shed some more light on the details of reproduction tactics of *E. maderensis*, since it has been observed that *Ericaceae* species are more prone to reproduce vegetatively after disturbances, such as fires (VALBUENA & VERA, 2002; PAULA & OJEDA, 2006, 2009). A research conducted by PAULA & OJEDA (2009), who studied the aboveground and belowground response of three species of *Erica*, *E. australis* L., *E. scoparia* L., and *E. arborea* L., and their capacity to resprout after disturbances revealed that the belowground (root) starch reserves are more crucial for resprouting when compared to the aboveground biomass.

Genista tenera propagation tests showed that this species seeds requires hot water scarification (5 min) treatment to break physical dormancy imposed by a hard seedcoat. Such treatment resulted in effective germination (between 66.7 and 91.6 %) since the heat was used to soften the seedcoat. These conditions mimicked high temperatures experienced by the plant during the summer reproductive period. Similar results have been obtained with *Genista* species, namely *Genista monspessulana* (L.) L. Johnson, where heat-treated seeds with boiling water germinated rapidly with over 50% of germination (ZOUHAR, 2005). A study performed on several species of the *Genistae* tribe showed that in general, the germination success was improved for nearly all of the *taxa* when scarifying agents (such as sulphuric acid) were applied (LOPEZ *et al*., 1999), substantiating the need for scarifying in order to break dormancy of these species. On the other hand, vegetative propagation proved to be an ineffective reproductive strategy for *G. tenera*.

Teline maderensis seed propagation tests showed a great similarity with the results obtained for *G. tenera*, with the seeds requiring hot water scarification (5 min) to remove physical dormancy. As with *G. tenera*, vegetative propagation of *T. maderensis* species was also unsuccessful, perhaps due to these species vegetative regeneration strategy. Species of the Fabaceae usually reproduce by vegetative means sporadically, after being subjected to a disturbance such as a fire, when typically the upper part of the plant is destroyed, and thus allowing for the vegetative reproduction to be initiated from the neck or base of the root system (SAHA & HOWE, 2003; ZOUHAR, 2005; REYES *et al*., 2009). This has been observed in other species of the *Genistae* tribe such as *Genista triacanthos* Brot., *Ulex minor* Roth and *Ulex europaeus* (L.) (REYES *et al*., 2009). Therefore, cuttings from their branches would hardly lead to a rooted cutting. This is in agreement with the observation that *G. tenera* and *T. maderensis* seeds germinated better under high temperature conditions.

Helichrysum melaleucum shows to have low seed productivity, and few viable seeds were found in nature. We observed that seeds of this species were heavily affected by pest feeding on the inflorescence and seeds, making seed availability scarce. The 0% germination obtained in seed trials without treatments can be explained by the seeds non–viability, or the need to combine factors or treatments to break their dormancy. Thus far, there has not been any attempt to propagate this species.

Studies conducted with species belonging to the same genus, *Helichrysum gossypinum* Webb., a Canary Island endemic, have shown that germination of this species could be mainly influenced by temperature (MAYA & PONCE, 1989). Another study with *H. gossypinum* and *Helichrysum monogynum* Burtt & Sunding, another endemic species of the Canary Islands, showed that without any seed treatment, only the latter species showed 20.0% germination (MAYA *et al*., 1988). In the same study, the use of GA_3 leads to germination of 27.5% of *H. gossypinum* and 12.5% of *H. monogynum* seeds (MAYA *et al*., 1988). However, it has been observed in nature and the gardens that seeds of this species sprout near the mother plant. We hypothesize that the mother plant could establish some interactions with soil rhizosphere, thus increasing the seed germination success. At the same time, vegetative propagation was mediocre, with 60% rooting success in the presence of KIBA (500 ppm). Vegetative propagation of *H. melaleucum* appears to be dependent on the time of year, as it has been already shown by DRAGOVIC (2005a; 2005b; 2009) in other *Helichrysum* species of Madeira. Therefore, it appears that the best propagation method of this species would be vegetative propagation due to its low seed germination.

Pericallis aurita seeds required mechanical scarification treatment to break seed dormancy in order to reach of 29.5% germination success, thus displaying physical dormancy, probably due to a hard seedcoat. Interestingly, a study performed by BAÑARES *et al*. (2003) with the germination of *Pericallis hansenii* (Kunkel) Sunding, a Canary endemic closely related to *P. aurita*, showed very different results. In these trials, the control tests resulted in 59.5% germination, whilst germination augmented with the hormone treatment (GA_3) reached 84%. However, since the germination success of *P. aurita* is fairly low, the plant could rely on vegetative propagation as a reproductive strategy. Simple stem cuttings and trench layering, regardless of the use of hormones can reach 90.0% of rooting success. Rooting success seems to have a physiological basis and is season dependent (time of year conditioned). Similarly, the study performed by BAÑARES *et al*. (2003) showed good vegetative propagation results with simple cuttings with the use of IBA 0.10% (80.0% rooting success).

Sideritis candicans does not require any sort of seed treatment to achieve high germination success, regardless of time of year, light conditions and cold stratification. On the other hand, a study performed with two Iberian *Sideritis* species, *Sideritis pungens* Benth. and *Sideritis chamaedryfolia* Cav. showed that germination of these species is affected by temperature, pre-treatment used, and especially light conditions (ESTRELLES *et al*., 2010). The different needs of *S. candicans* could be due to the diverse environments of the species, since *S. pungens* grows in sub–humid high mountains (1,000 – 2,000 m a.s.l.) and *S. chamaedryfolia* is found in dry, thermo–mesomediterranean habitats (500 – 850 m a.s.l.) (ESTRELLES *et al*., 2010). Likewise, when KADIS *et al*. (2010) studied the germination requirements of *Sideritis cypria* Post, it was shown that temperature was the main factor impacting this species germination, but no additional pre–treatments were required, as in *S. candicans*. In contrast, a study conducted in Bulgaria showed that a species of *Sideritis*, *Sideritis scardica* Griseb., required GA_3 to germinate (80.9%), whilst seeds with no treatment did not germinate at all (KOZUHAROVA, 2009). This plant also shows an effective vegetative propagation, most efficient, when simple cuttings are combined with a 5 second quick-dip in 1000 ppm of IBA (70 – 75%), preferably in late spring – early summer.

The statistical tests generated results that are in contrast with the field observations, showing that there was no statistical difference between treatments, both in seed propagation and vegetative propagation. These divergences between field results and statistical results could be due to the use of samples to small or insufficient to detect any effect in statistical tests, i.e., the trials and their effects were with an inadequate sample size, thus providing a false negative (KANYONGO *et al*., 2007; RITCHIE *et al*., 2011; REINHART, 2012; COLQUHOUN, 2014).

Overall, physical dormancy appears to be the most common type of dormancy displayed by the nine native Madeiran species studied in the present work. At the same time, the major ecological constraints to seed germination are represented by temperature and water availability. Physical dormancy of hard seedcoat species (*A. lemanniana, C. canariensis, G. tenera* and *T. maderensis*) appears to be mainly due to inability of the seeds to imbibe water.

We were able to verify that the mechanical scarification is a suitable method for *A. lemmaniana* and *P. aurita,* while *G. tenera* and *T. maderensis* require hot water scarification for 5 min. On the other hand, *A. maderensis* requires cold stratification and *C. canariensis* could be propagated either by the mechanical scarification or cold water immersion. Interestingly *S. candicans* does not require any seed treatment to germinate, even though the application of GA_3 also gave positive results. The only species that did not show any germination was *H. melaleucum*, in which the lack of viable seeds led to insufficient germination trials. Therefore, sexual propagation of this species requires further studies.

Vegetative propagation appears to be a method applicable to only a few species with rooting success higher than 50% in only three species, namely *H. melaleucum, P. aurita* and *S. candicans*, followed by *C. canariensis*, reaching near 50 % success. All tested species adopted different vegetative regeneration strategies – *C. canariensis* does not require rooting agents and is propagated by simple stem cuttings, *H. melaleucum* requires KIBA (500 ppm) and propagates better in the summer, *P. aurita* is best propagated by the trench layering, and *S. candicans* with a 5 sec. dip in IBA 1,000 ppm.

Acknowledgments

José Manuel Rodrigues and other staff from the Ecological Park of Funchal, Sónia Ferraz, Marta Moreira and Filipe Ganança from the ISOPlexis Genebank, and the staff of the Floriculture Centre. We thank Jan J. Slaski of the ISOPlexis Genebank and the Alberta Innovates Technology Futures for reviewing the manuscript.

Abbreviations used: m - meters; a.s.l. - above sea level; GA_3 - gibberellic acid; IBA - indole-3-butyric acid; KIBA - potassium salt of indole-3-butyric acid; IBA 0.1% - powder formulation of 0.1% w/w IBA; IBA 0.3% - powder formulation of 0.3% p/p IBA; IBA 0.8% - powder formulation of 0.8% w/w IBA; ppm - parts-per-million (10^{-6}), h – hour(s); min - minute(s); sec - second(s); T_{50} - number of days to germination of 50% of all germinated seeds; N - final number of emergence; N_1 - seeds germinated when $N_1 < N/2$; N_2 - seeds germinated when $N/2 < N_2$; T_1 time $N_1 < N/2$; T_2 – time $N/2 < N_2$.

References

AYANOĞLU, F., MERT, A., ERDOĞAN, C., KAYA, A., 2002. Propagation of Some Native Grown Medicinal Plants by Stem Cuttings. *J Herbs Spices Med Plants* **9**(4): 405 - 411.

BACCHETTA, G., SÁNCHEZ, A.B., FENU, G., JIMÉNEZ-ALFARO, B., MATTANA, E., PIOTTO, B., VIREVAIRE, M. (EDS), 2008. *Conservación ex situ de plantas silvestres*. Principado de Asturias/La Caixa. 378 pp.

BAÑARES, A., MARRERO, M., CARQUÉ, E., FERNÁNDEZ, A., 2003. Plan de Recuperación de la Flora Amenazada del Parque Nacional de Garajonay. La Gomera (Islas Canarias). Germinación y Restituciones de *Pericallis hansenii, Gonospermum gomerae* e *llex perado* ssp. *lopezlillai*. *Bot. Macaronésica* **24**: 3-16.

BILZ, M., KELL, S.P., MAXTED, N., LANSDOWN, R.V., 2011. *European Red List of Vascular Plants*. Publications Office of the European Union, Luxembourg. 130 pp.

BONNER, F.T., KARRFALT, R.P. (Eds.), 2008. The Woody Plant Seed Manual. Agriculture Handbook No. 727. U.S. Department of Agriculture, Forest Service, Washington DC., USA. 1223 pp.

BORGES, P.A.V., ABREU, C., AGUIAR, A.M.F., CARVALHO, P., FONTINHA, S., JARDIM, R., MELO, I., OLIVEIRA, P., SEQUEIRA, M.M., SÉRGIO, C., SERRANO, A.R.M., SIM-SIM, M., VIEIRA, P., 2008. Terrestrial and freshwater biodiversity of the Madeira and Selvagens archipelagos. In: BORGES, P.A.V., ABREU, C, AGUIAR, A.M.F., CARVALHO, P., JARDIM, R., MELO, I., OLIVEIRA, P., SÉRGIO, C., SERRANO, A.R.M., VIEIRA, P. (Eds), A list of the terrestrial fungi, flora and fauna of Madeira and Selvagens archipelagos. Direcção Regional do Ambiente da Madeira and Universidade dos Açores, Funchal and Angra do Heroísmo. pp. 13-25.

CAPELO, J., SEQUEIRA, M.M., JARDIM, R., COSTA, J.C., 2004. Guia da Excursão Geobotânica dos V Encontros ALFA 2004 à Ilha da Madeira. In: CAPELO, J., (Ed.) A paisagem vegetal da ilha da Madeira. *Quercetea* **6**. pp: 5-46.

CERVELLI, C., 2009. Introduzione di nuove specie di *Helichrysum* per la coltivazione di piante in vaso. *Flortecnica* **4**: 58-65.

COLQUHOUN, D., 2014. An investigation of the false discovery rate and the misinterpretation of *p*-values. *R. Soc. Open Sci.* **1**: 140216. Available in http://dx.doi.org/10.1098/rsos.140216. [16 May 2015].

CORREIA, A.V., TAVARES, M.T., PEREIRA, J.S., 2006. Florestas. In: SANTOS, F.D., AGUIAR, R. (Eds). Impactos e Medidas de Adaptação às Alterações Climáticas no Arquipélago da Madeira – Projecto CLIMAAT II Funchal, Direcção Regional do Ambiente da Madeira. pp: 52 – 59. Available in http://www.sra.pt/files/PDF/Destaques/Brochura%20 CLIMAAT_II_MadeiraFINAL.pdf. [24 February 2014].

CRUZ, M.J., AGUIAR, R., CORREIA, A., TAVARES, T., PEREIRA, J.S., SANTOS, F.D., 2009. Impacts of climate change on the terrestrial ecosystems of Madeira. *Int J Des Nat Ecodyn* **4**(4): 413-422.

DORNER, J., 2002. An introduction to using native plants in restoration projects. Plant Conservation Alliance. Available in http://www.nps.gov/plants/restore/pubs/ intronatplant/intronatplant.pdf. [20 March 2012].

DOUSSI, M.A., THANOS, C.A., 1993. The ecophysiology of fire-induced germination in hard-seeded plants. In CÔME, D., CORBINEAU, F. (Eds), Fourth International Workshop on Seeds, Basic and Applied Aspects of Seed Biology, July 20-24, 1992. Angers, France. Vol. 2. ASFIS. Paris: 455-460.

DOUSSI, M.A., THANOS, C.A., 1994. Post-fire regeneration of hardseeded plants: ecophysiology of seed germination. Proceedings of the *2nd International Conference on Forest Fire Research* Vol. II, Coimbra (Portugal), November 21-24. pp. 1035-1044.

DRAGOVIC, M.J.O., 2005a. Utilization of *Helichrysum* Devium Johns. and *Helichrysum obconicum* Dc. as Ornamental Plants. ISHS 5th International Symposium on New Floricultural Crops, *Acta Horticulturae* **683**: 181-187.

DRAGOVIC, M.J.O., 2005b. Flowering behavior of *Helichrysum obconicum* DC. ISHS 5th International Symposium on New Floricultural Crops, *Acta Horticulturae* **683**: 357-361.

DRAGOVIC, M.J.O., 2009. Propagation of *Helichrysum obconicum* DC. and *Helichrysum devium* Johns. from cuttings. SHS 6th International Symposium on New Floricultural Crops, *Acta Horticulturae* **813**: 547-552.

ELLIS, R.H., HONG, T.D., ROBERTS, E.H., 1985. Handbook of seed technology for genebanks, Volume II: compendium of specific germination information and test recommendation. Handbooks for Genebanks, 3. International Board for Plant Genetic Resources (IBPGR). Rome, Italy. 456 pp. Available in http://www. bioversityinternational. org /e-library /publications/detail/handbook-of-seed-technology-for-genebanks/. [20 March 2011].

ENSCOBASE: the ENSCONET Virtual Seed Bank (2011) *Cedronella canariensis* germination tests. Available in http://enscobase.maich.gr/germlistpercent.tml? Autocomplete _parameter =Cedronella+canariensis. [3 May 2011].

ESTRELLES, E., ALBERT, F., NAVARRO, A., PRIETO, J., IBARS, A.M., 2004. Germination behaviour of Labiatae SW distributed in the Iberian Peninsula. 4th European Conference on the conservation of wild plants, Planta Europa IV Proceedings, Valencia (Spain), September 17-20. Available in http://www.nerium.net/ plantaeuropa/Download/Procedings/Estrelles_Albert_et_al.pdf. [20 May 2011].

ESTRELLES, E., GÜEMES, J., RIERA, J., BOSCAIU, M., IBARS, A.M., COSTA, M., 2010. Seed Germination Behaviour in *Sideritis* from Different Iberian Habitats. *Not Bot Horti Agrobot Cluj Napoca* **38**(1): 09-13.

EUROPEAN COMMISSION DG ENVIRONMENT, 2013. Interpretation Manual of European Union Habitats, EUR 28. Available in http://ec.europa.eu/environment/nature/ legislation/habitatsdirective/index_en.htm. [5 January 2014].

FAGÚNDEZ, J., JUAN, R., FERNÁNDEZ, I., PASTOR, J., IZCO, J., 2009. Systematic relevance of seed coat anatomy in the European heathers (Ericeae, Ericaceae). *Plant Syst Evol* **284**(1): 65-76.

FARIA, B.F., ABREU, C., AGUIAR, A.F., AUGUSTO, J., JARDIM, R., LOBO, C., OLIVEIRA, P., TEIXEIRA, D., 2008. La perspectiva archipelágica: Madeira. In: MARTÍN, J.L., ARECHAVALETA, M., BORGES, P.A.V., FARIA, B. (Eds). Top 100. Las 100 especies amenazadas prioritarias de gestión en la región europea biogeográfica de la Macaronesia Consejería de Medio Ambiente y Ordenación Territorial. Gobierno de Canarias. pp. 109-128.

FREITAS, S., COUTINHO, M., 2006. Biodiversidade. In: SANTOS, F.D., AGUIAR, R. (Eds), Impactos e Medidas de Adaptação às Alterações Climáticas no Arquipélago da Madeira – Projecto CLIMAAT II Direcção Regional do Ambiente da Madeira, Funchal. pp: 52 – 59. Available in http://www.sra.pt/files/PDF/Destaques/ Brochura%20CLIMAAT_II_MadeiraFINAL.pdf._[24 February 2014].

GARCÍA, A.D., FARIA, C., ALMEIDA, M.H., VIZCAÍNO, E.D., 2005. Efeito da aplicação de diferentes tratamentos na germinação e reprodução vegetativa de Ericáceas. In: *Actas do 5° Congresso Florestal Nacional: A Floresta e as Gentes*, Sociedade Portuguesa de Ciências Florestais, Viseu (Portugal), May 16-19. pp. 1–11.

GENMEDOC, 2004-2006. Prácticas de germinación en los bancos de semillas de la red GENMEDOC. Proyecto Interreg IIIB Medocc. Available in http://www.semclimed. org/ allegati/74.pdf. [12 March 2011].

HANLEY, M.E., FENNER, M., 1998. Pre-germination temperature and the survivorship and onward growth of Mediterranean fire-following plant species. *Acta Oecol* **19**(2): 181-187.

HARTMANN, H.T., KESTER, D.E., DAVIES, F.T., GENEVE, R.L., 2011. Hartmann and Kester's Plant Propagation - Principles and Practices. 8th Edition. Prentice Hall. Upper Saddle River, New Jersey, USA. 915 pp.

HEIMBINDER, E., 2001. Revegetation of a San Francisco coastal salt marsh. *NPJ* **2**(1): 54-59.

IBAÑEZ, A.N., PASSERA, C.B., 1997. Factors affecting the germination of albaida (*Anthyllis cytisoides* L.), a forage legume of the Mediterranean coast. *J Arid Environ* **35**: 225-231.

JARDIM, R., FERNANDES, F., CARVALHO, J., 2006. Flora Vascular. In: FARIA, B.F., JARDIM, R., (Coords). Fauna e Flora da Madeira. Espécies endémicas ameaçadas: vertebrados e flora vascular Governo Regional da Madeira, DRAamb, Madeira. pp. 39-135.

JARDIM, R., FRANCISCO, D., 2000. Flora Endémica da Madeira. Múchia Publicações. Funchal, Madeira, Portugal. 339 pp.

JARDIM, R., SEQUEIRA, M.M., 2008. The vascular plants (Pteridophyta and Spermatophyta) of the Madeira and Selvagens archipelagos. In: BORGES, P.A.V., ABREU, C., AGUIAR, A.M.F., CARVALHO, P., JARDIM, R., MELO, I., OLIVEIRA, P., SÉRGIO, C., SERRANO, A.R.M., VIEIRA, P. (Eds). A list of the terrestrial fungi, flora and fauna of Madeira and Selvagens archipelagos Direcção Regional do Ambiente da Madeira, and Universidade dos Açores, Funchal and Angra do Heroísmo. pp. 157-178.

JARDIM, R., SEQUEIRA, M.M., CAPELO, J., 2007. Biologia e ecologia das florestas das ilhas – Madeira. In: SILVA, J., (Ed). Árvores e Florestas de Portugal. Volume 6, Açores e Madeira - A Floresta das Ilhas Edição da Fundação Luso Americana para o Desenvolvimento, Público e Liga para a Protecção da Natureza, Lisboa. pp. 255 - 296.

KADIS, C., KOUNNAMAS, C., GEORGHIOU, K., 2010. Seed germination and conservation of endemic, rare, and threatened aromatic plants of Cyprus. *Isr. J. Plant Sci.* **58**: 251-261.

KANYONGO, G.Y., BROOK, G.P., KYEI-BLANKSON, L., GOCMEN, G., 2007. Reliability and Statistical Power: How Measurement Fallibility Affects Power and Required Sample Sizes for Several Parametric and Nonparametric Statistics. *Journal of Modern Applied Statistical Methods* **6**(1): 81-90.

KOZUHAROVA, E., 2009. New *Ex Situ* Collection of Rare and Threatened Medicinal Plants in the Pirin Mts. (Bulgaria). *Ekoloji* **18**(72): 32-44.

LOPES, S., FARIA, C., TELHADA, A.E., ALMEIDA, M.H., 2005. Contribuição para a propagação vegetativa de algumas espécies arbustivas autóctones da região algarvia. In: Actas do 5° Congresso Florestal Nacional: A Floresta e as Gentes. Sociedade Portuguesa de Ciências Florestais, Viseu (Portugal), May 16-19. Available in http://www.repository.utl.pt/handle/10400.5/703. [26 March 2011].

LOPEZ, J., DEVESA, J.A., RUIZ, T., ORTEGA-OLIVENCIA, A., 1999. Seed germination in *Genisteae* (Fabaceae) from South-West Spain. *Phyton* **39**(1): 107-129.

LUNA, T., WILKINSON, K., DUMROESE, R.K., 2009. Chapter 8: Seed germination and sowing options. In: DUMROESE, R.K., LUNA, T., LANDIS, T.D., (Eds). Nursery manual for native plants: A guide for tribal nurseries - Volume 1: Nursery management, Agriculture Handbook 730. U.S. Department of Agriculture, Forest Service, Washington, D.C. pp. 133-151.

MAYA, P., MONZON, A., PONCE, M., 1988. Datos sobre la germinacion de especies endemicas canarias. *Bot. Macaronésica* **16**: 67-80.

MAYA, P., PONCE, M., 1989. Algunos datos sobre la interacción entre luz y temperatura en la germinación de algunas especies de Asteraceaes endémicas de Canarias. *Bot. Macaronésica* **17**: 15-26.

MESQUITA, S., CAPELO, J., SOUSA, J., 2004. Bioclimatologia da Ilha da Madeira: abordagem numérica. In: CAPELO, J., (Ed). A paisagem vegetal da Ilha da Madeira. *Quercetea* **6**: 47-59.

MEYER, S.E., 2006. Strategies for Seed Propagation of Native Forbs. In: RILEY, L.E., DUMROESE, R.K., LANDIS, T.D., (Tech. Coords), National Proceedings: Forest and Conservation Nursery Associations - 2005. Proc. RMRS-P-43 U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO. pp: 3-9. Available in http://www.rngr.net/publications/proceedings/2005/strategies-for-seed-propagation-of-native-forbs/?searchterm=Strategies for Seed Propagation of Native Forbs. [24 February 2014].

MIRANDA, P.M.A. (Coord.), VALENTE, M.A., TOMÉ, A., TRIGO, R., COELHO, M.F., AGUIAR, A., AZEVEDO, E.B., 2006. O clima de Portugal nos séculos XX e XXI. In: Alterações Climáticas em Portugal. Cenários, Impactos e Medidas de Adaptação - Projecto SIAM II (Santos FD, Miranda P, eds). Gradiva, Lisboa. pp. 45-113.

MORBIDONI, M., ESTRELLES, E., SORIANO, P., MARTÍNEZ-SOLÍS, I., BIONDI, E., 2008. Effects of environmental factors on seed germination of *Anthyllis barba-jovis* L. *Plant Biosyst* **142**(2): 275-286.

MYERS, N., MITTERMELER, R.A., MITTERMELER, C.G., FONSECA, G.A.B., KENTS, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853-858.

OLIVEIRA, R.P., RIBEIRO, L., NASCIMENTO, J., AMARO, S., 2006. Recursos Hídricos. In: SANTOS, F.D., AGUIAR, R., (Eds), Impactos e Medidas de Adaptação às Alterações Climáticas no Arquipélago da Madeira – Projecto CLIMAAT II. Direcção Regional do Ambiente da Madeira, Funchal. pp: 34 – 51. Available in http://www.sra.pt /files/PDF/Destaques/Brochura%20CLIMAAT_II_MadeiraFINAL.pdf. [24 February 2014].

PAPAFOTIOU, M., KALANTZIS, A., 2009. Seed germination and *in vitro* propagation of *Sideritis athoa*. *Acta Hort. (ISHS)* **813**: 471-476. Available in http://www.actahort. org/books/813/813_62.htm [30 December 2011].

PAULA, S., OJEDA, F., 2006. Resistance of three co-occurring resprouter *Erica* species to highly frequent disturbance. *Plant Ecol*. **183**(2): 329–336.

PAULA, S., OJEDA, F., 2009. Belowground starch consumption after recurrent severe disturbance in three resprouter species of the genus *Erica*. *Botany* **87**(3): 253–259.

PRESS, J.R., SHORT, M.J., 1994. Flora of Madeira. The Natural History Museum, HMSO, London, England. 574 pp.

PRIETO, J., ESTRELLES, E., IBARS, A.M., 2004. *Anthyllis lagascana*: from seed to adult plant. *Ex situ* conservation and introduction experiences. Proceedings of the 4th European Conference on the Conservation of Wild Plants, Planta Europa, Valencia (Spain) September 17-20. Available in http://www.nerium.net/plantaeuropa/Download/ Procedings/Prieto_et_al.pdf. [13 April 2011].

REINHART, A., 2012. Statistics Done Wrong. Available at http://www.refsmmat. com/files/statistics-done-wrong.pdf. [18 May 2015].

REYES, O., CASAL, M., REGO, F.C., 2009. Resprouting Ability of Six Atlantic Shrub Species. *Folia Geobot*. **44**: 19-29.

RITCHIE, G.L., WHITAKER, J.R., COLLINS, G.C., 2011. Effect of Sample Size on Cotton Plant Mapping Analysis and Results. *The Journal of Cotton Science* **15**: 224-232.

SAHA, S., HOWE, H.F., 2003. Species composition and fire in a dry deciduous forest. *Ecology* **84**: 3118-3123.

SAUTER, R., BRINK, P.T., WITHANA, S., MAZZA, L., PONDICHIE, F., with contributions from CLINTON, J., LOPES, A., BEGO, K., 2013a. Impacts of climate change on all European islands. A report by the Institute for European Environmental Policy (IEEP) for the Greens/EFA of the European Parliament. Final Report. Brussels. Available in http://www.ieep.eu/publications/2013/11/impacts-of-climate-change-on-european-islands. [13January 2014].

SAUTER, R., BRINK, P., WITHANA, S., MAZZA, L., PONDICHIE, F., LOPES, A., CLINTON, J., BEGO, K., 2013b. Five case studies on the impacts of climate change on European islands, Annex II to the final report 'Impacts of climate change on all European islands' by the Institute for European Environmental Policy (IEEP) for the Greens/EFA of the European Parliament. Draft Final Report. Brussels. Available in http://www.ieep.eu/publications/2013/11/impacts-of-climate-change-on-european-islands. [13 January 2014].

SERRANO-BERNARDO, F., ROSÚA, J.L., DÍAZ-MIGUEL, M., 2007. Light and Temperature effects on seed germination of four native species of Mediterranean high mountains (Spain). *Phyton* **76**: 27-38.

TAIZ, L., ZEIGER, E., 2010. Plant Physiology, 5Th Edition (International Edition). Sinauer Associates, Sunderland, MA., USA. 912 pp.

THANOS, C.A., DOUSSI, M.A., 1995. Ecophysiology of seed germination in endemic labiates of Crete. *Isr. J. Plant Sci.* **43**: 227-237.

UÇAR, E., TURGUT, K., 2011. E-Posters: *In Vitro* Regeneration of Mountain Tea (*Sideritis stricta*). 7th International Symposium on *In Vitro* Culture and Horticultural Breeding Biotechnological advances, Ghent (Belgium), September 18-22. pp. 18-22.

VALBUENA, L., VERA, M.L., 2002. The effects of thermal scarification and seed storage on germination of four heathland species. *Plant Ecol.* **161**: 137-144.

ZOUHAR, K., 2005. *Genista monspessulana*. In: Fire Effects Information System [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available in http://www.fs.fed.us/database/feis/. [21 March 2011].

Entregue para publicação em setembro de 2015
Aceite para publicação em maio de 2017