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Morphophysiological dormancy and germination in seeds of the Azorean tree *Picconia azorica*

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Summary

Picconia azorica (Oleaceae) is an Azorean tree with high ecological value. We investigated possible causes for *P. azorica* seed dormancy and the germination conditions to break it, using acid scarification (scarified stones) or complete removal of the endocarp (seeds), in conjunction with different stratification and incubation temperature regimes and gibberellic acid (GA_3) treatments. Embryos in ripe drupes were subspatulate, axile and occupied 60% of the endosperm length. Water imbibition was verified for both acid scarified stones and seeds. The highest total phenolic compounds content occurred in the seed coat (36.4 ± 1.51 mg GAE g^{-1} FW). Germination was significantly affected by the type of endocarp treatment (acid scarification, 23%; removal of the endocarp, 46%), and by temperature (62% at 10/5°C and 15/10°C; 8% at 20/15°C and 4% 25/20°C), but not by concentration of GA_3. Under the two best temperature regimes, only acid scarified stones were significantly affected by the stratification regime, with the highest germination (ca. 60%) after 60 days cold or 30 days warm followed by 30 days cold stratification regimes, although always lower than those obtained for seeds (> 80%). Epicotyl development required low temperature and three months to cotyledon leaf expansion. Germination requirements and embryo characteristics suggest a non-deep simple epicotyl morphophysiological dormancy including the occurrence of a possible chemical inhibition mechanism. For *P. azorica* propagation we recommend using naked seeds incubated at a temperature of 10/5 or 15/10°C.

Introduction

Picconia azorica (Tutin) Knobl. (Oleaceae), locally named "Pau-branco", is an endangered species endemic to the Azores Archipelago, where it is found in all the islands except Graciosa (Cardoso *et al.,* 2008; Silva *et al.,* 2009; Silva *et al.,* 2010). *P. azorica* is an evergreen shrub or small tree growing up to 8 m tall, with simple, lanceolate to ovate, opposite leaves with entire margins. It is found in coastal and medium altitude forests, in coastal cliffs, ravines, lava flows, coastal scrubland [with *Erica azorica* Hochst. ex Seub.,

Morella faya (Aiton) Wilbur or both] and in natural forests with *M. faya* or *Laurus azorica* (Seub.) Franco (Silva *et al.,* 2009). Flowering occurs from March to July with small white flowers developing in axillary clusters. Fruits are dark blue drupes, about 1.5 cm long, which become ripe from July to the end of October. Long periods of overlapping flowering and fructification occur, with flowers and ripe fruits occurring concomitantly in the same individual. In spring, it is common to observe in the soil below the adult trees, seedlings (approximately 1 m^{-2}), about 4 to 8 cm long with the first leaf pairs and also newly germinated seeds still showing a partially decayed endocarp.

P. azorica was historically used in the Azores for furniture construction and religious statuary. It is a priority Azorean endemic species for conservation, listed as endangered (EN B1 + 2c) on the IUCN Red List 2004, and in Annex II of the EC Habitats Directive, due to over-exploitation, habitat degradation, expansion of agricultural land, forestation, competition by alien species and isolation of populations (Cardoso *et al.,* 2008; Silva *et al*., 2009). Clearly, recovery of *P. azorica* populations requires propagation measures, particularly in those islands where its populations are more depauperate. Recently, Martins *et al.* (2011) established an efficient protocol for the propagation of this species by air-layering. However, in order to maintain the genetic variability of small size populations, propagation by seeds might be more appropriate.

There have been many seed germination studies in the Oleaceae family (in *Olea europaea* L. and *Fraxinus* L. spp.) and the occurrence of dormancy is well documented (Ellis *et al.,* 1985; Baskin and Baskin, 2005). Sotomayor-Leòn and Caballero (1994), in an olive study, reported that seed dormancy was imposed by the endocarp. High germination rates were achieved using olive seeds without the endocarp (90%) or stones scarified with sulphuric acid (70%) for 5-10 min (Crisosto and Sutter, 1985; Vachkoo *et al.,* 1993; Bandino *et al.*, 1999). Cold stratification is also effective for overcoming dormancy in *O. europaea* (Voyiatzis, 1995). For *Fraxinus*, a genus phylogenetically close to *Picconia* (Ferreira *et al.,* 2010), it was found that one month warm followed by one to three months cold, or cold stratification alone, resulted in successful germination (Steinbauer, 1937; Piotto *et al.*, 2003; Bonner, 2001; Tilki and Cicek, 2005), while more than one month warm stratification was ineffective (Tilki and Cicek, 2005). Gibberellic acid improves the germination of some species of Oleaceae (Ashley, 2000; Hussain and Hussain, 2004), but not of others (Villiers and Wareing, 1964; Arrillaga *et al.*, 1992) and chemical inhibitors present in the seed coat have been reported (Vachkoo *et al.,* 1993; Fabbri *et al.,* 2004).

There is only one study that reports the successful propagation of *P. azorica*, using stones, with 48% emerged seedlings, three to four months after sowing in an outdoor nursery, with a fungicide pre-treatment. Stones were sown three to four months after their natural dispersal time, in a different location than the site of growth (Fagundo and Isidoro, 2004). Earlier studies with this species indicated the occurrence of seed dormancy (Maciel, 1995, 1996), coupled with a difficult development of the cotyledon leaves which led to necrosis of all the germinated seeds (GB Maciel, University of Azores, Portugal, 'pers. comm.'). We also performed preliminary germination tests with stones of *P. azorica* without scarification, using different temperatures and hormone concentrations, which were largely unsuccessful (unpublished data). This research aims to improve our understanding about seed germination mechanisms in *P. azorica* and to establish an efficient propagation

protocol. We analysed embryo morphometric data, seed water imbibition, seed viability and tested some of the factors that might promote the germination of *P. azorica* seeds, namely removal of the endocarp, chemical scarification of the stone, different periods of cold or warm followed by cold stratification, treatment with gibberellic acid, and different alternating temperature regimes. Total phenolic content in fruits was also determined, to evaluate the possible existence of chemical inhibitors of germination.

Materials and methods

Sampling and treatment of fruits

Ripe drupes were harvested at the end of September, in São Miguel Island, Azores, at the only existing population, located at Lombo Gordo, in the southeast coast of the island. Shriveled and unusually small fruits were rejected. The exocarp and mesocarp were removed in all the fruits and stones washed under running tap water.

Embryo characteristics, water imbibition, seed viability and total phenolic content

In order to evaluate the developmental stage of the embryo, total seed length and embryo length (including primordial leaves) were measured on a sample of 90 randomly selected seeds, using a digital calliper (± 0.02 mm) and a stereomicroscope.

Water imbibition was determined using two sets of 3×30 intact dry seeds (seeds with endocarp completely removed) or stones (seeds enclosed by the endocarp), which were weighed, immersed in distilled water and reweighed after 1, 6, 12 and 24 hours. Seed viability was determined by tetrazolium test (AOSA, 2000), using 3×30 seeds and 3×30 acid-scarified stones, using the same scarification methodology as used for seed germination, to evaluate if the embryo was affected by the acid. To determine the phenolic content, finely powdered fruit pulp, endocarp, seed coat or endosperm plus embryo, was stirred with methanol at room temperature for 16 hours. The extracts were filtered, concentrated to dryness and stored at -20°C until further analysis. The remaining material was then extracted with boiling water for five minutes, filtered, concentrated to dryness and stored. Total phenolic compounds (TPC) in the resulting eight plant extracts was determined by using the Folin-Ciocalteu colorimetric methodology previously described by Rainha *et al.* (2011). TPC in plant extracts is expressed in gallic acid equivalents (GAE) as a function of the fresh weight (FW).

Seed germination

Germination tests were done in growth chambers with automatic temperature control (error margin of ca. 1°C) and a light period of 12 hours per day, provided by six fluorescent lamps with a photosynthetic photon flux density (PPFD) of 19-22 μmol m^{-2} s^{-1}. Scarified stones/seeds were placed on filter paper in 12 cm diameter Petri dishes sealed with Parafilm. To control fungal contamination, stones/seeds were periodically washed, transferred to clean Petri dishes and the filter paper was always fully moistened with a 6% (w/v) Benomyl solution.

Since, in preliminary germination tests using stones, necrosis was observed in the majority of the seeds after one year (unpublished results), and also considering the information available in the literature, we decided to include two stone treatments: i) manual removal of the endocarp using a stainless steel nipper; and ii) 15 minutes scarification in sulphuric acid. For the latter, preliminary essays were performed to find the best immersion time (to thin the endocarp without damaging the seed).

Scarified stones/seeds were stratified 30, 60 or 90 days at 4°C, or 30 days at 20°C followed by 30, 60 or 90 days at 4°C before the germination test. A control was included without stratification. Sets of 360 scarified stones/seeds were placed inside net bags in plastic trays (40 × 20 cm), using a mixture of moist perlite and peat (1:1) as substrate, and a refrigerator for cold stratification or a temperature controlled chamber for warm stratification. During the stratification process, randomly selected seeds were transversally cut and embryo development visually checked.

As reviewed by Baskin and Baskin (1998), alternating temperatures are usually more favourable for germination. Thus, four alternating temperature regimes were used, 10/5, 15/10, 20/15 and 25/20°C, based on the mean temperatures for each season of the year, estimated for *P. azorica* natural habitats by the CIELO model (Azevedo, 1996).

To evaluate the effect of gibberellic acid, scarified stones/seeds were immersed in water (control) or in four different solutions of GA_3 (100, 250, 500 and 750 mg l^{-1}) for 72 hours. After immersion, stones/seeds were placed on filter paper to dry the excess of hormone solution.

Based on the four studied factors, type of stone treatment (two levels), stratification (seven levels), temperature (four levels) and growth regulator concentration (five levels), a fully factorial design was used, resulting in a total of 280 different treatments, with three Petri dishes per treatment and 30 scarified stones/seeds per dish. In those cases where some of the seeds germinated during the stratification process, the remaining seeds were distributed evenly by the different treatments.

Germination, corresponding to visible radicle emergence (2-3 mm long), was checked weekly for 35 weeks (approximately eight months).

Seedling growth

Three replicates of 30 germinated seeds were maintained in Petri dishes with moistened filter paper under the same conditions as those for germination (10/5, 15/10, 20/15 and 25/20°C), to determine the best temperature regime for radicle development. Radicle length was measured after 30 days. After radicle development, 3 × 30 randomly selected germinated seeds were planted in moist peat cylinders and maintained inside transparent plastic containers in chambers at 10/5 and 25/20°C. The period of time to reach the full development of the cotyledon leaves was recorded.

Statistical analysis

Mean germination percentages were calculated for each treatment after 35 weeks of incubation. Percentages were transformed using ArcSin(SquareRoot(X)), following Zar (1999). Data normality was determined with the Kolmogorov-Smirnov test. Since transformed data followed the usual parametric tests assumptions (i.e. normality,

homoscedasticity), multifactor ANOVA was performed to test for main effects and interactions between the studied factors (type of stone treatment, stratification regime, growth regulator concentration, and incubation temperature). A Tukey HSD test was used as a multiple comparison procedure. ANOVA and Tukey test were also used when comparing germination rates during stratification (effect of the stratification regime and of the type of stone treatment) and for comparing radicle growth and survival in seedlings incubated at the four alternating temperature regimes. Statistical analyses were performed using SPSS 18.0 and Microsoft Office Excel 2003.

In relevant cases, namely those corresponding to higher germination rates, cumulative germination curves were fitted using the Gompertz sigmoid function (Gompertz, 1825; Laterra and Bazzalo, 1999; Moura and Silva, 2010). The estimated models were then used to estimate T_{50} values (i.e. the time taken for germination to reach 50%).

Results

Embryo characteristics, water imbibition, seed viability and total phenolic content

The subspatulate axile embryo (Martin, 1946), occupied an average 60% of the endosperm length, and presented slightly expanded cotyledons about 50% the full length of the embryo. Mean endosperm length was 8.7 ± 0.10 mm (mean ± s.e.) and embryo length was 5.9 ± 0.05 mm. The tetrazolium test showed a viability of close to 100% for embryos resulting from either scarified stones or seeds.

In scarified stones, water imbibition was faster during the first hour (19% increase in weight) and declined afterwards to less than 1% increase in weight per hour, with a total weight increase of 27% after 24 hours (data not shown). Endocarp alone increased from 0.036 to 0.038 g (6%). The weight of seeds increased from 0.041 to 0.060 g (46%) in 24 hours. The rate of imbibition was not as fast in the first hour as for scarified stones (6.7% increase in weight per hour), and further declined afterwards to values below 2% increase in weight per hour.

The seed coat had the highest content of phenolic substances followed by the endosperm plus embryo (table 1). The seed coat had approximately twice the amount of water soluble phenolic compounds, when compared with the endosperm plus embryo or with the external pericarp layers. The endocarp always showed the lowest phenolic content.

Table 1. Total phenolic content of different seed parts of *Picconia azorica* in Gallic acid equivalents (GAE).

	Total phenolic content (mg GAE g^{-1} fresh weight)			
Extract	Pulp	Endocarp	Seed coat	Endosperm + embryo
Methanolic	3.7 ± 0.14	4.2 ± 0.33	36.4 ± 1.51	14.4 ± 0.65
Hot water	12.3 ± 0.94	4.8 ± 0.21	26.9 ± 0.93	14.7 ± 0.88

Seed germination

A multifactorial ANOVA applied to the entire set of results showed that final germination was significantly affected by the type of endocarp treatment (acid scarification, 23% versus complete removal, 46%; $F = 765.5$; $P < 0.001$), by stratification ($F = 24$; $P < 0.001$) and by temperature regime ($F = 1449.1$; $P < 0.001$). There was no significant effect of GA_3 concentration on germination rate ($F = 0.82$; $P = 0.520$). Removal of the endocarp resulted in higher germination than acid scarification, with a global germination rate of 46% and 23%, respectively (figure 1).

Results of a Tukey test ($\alpha = 0.05$) applied after ANOVA showed that germination rate at 10/5 and 15/10°C (62.7%) was significantly higher than germination at 20/15 (8.0%) or 25/20°C (3.6%). Significant differences were only found between the two best stratification treatments (39.5% following 30 days cold; 40.9% following 30 days warm + 30 days cold) and the least effective treatment (27.6% following 90 days cold).

ANOVA of the data for the two lowest temperature regimes showed that the effect of stratification was significant for scarified stones ($F = 11.3$; $P < 0.001$), but not for seeds ($F = 2.1$; $P = 0.050$), the latter resulting in the highest germination (up to 86.2%). For scarified stones, the highest germination rates (59.7 - 65.8%) were obtained with the 60 days cold and warm 30 days + cold 30 days stratification treatments.

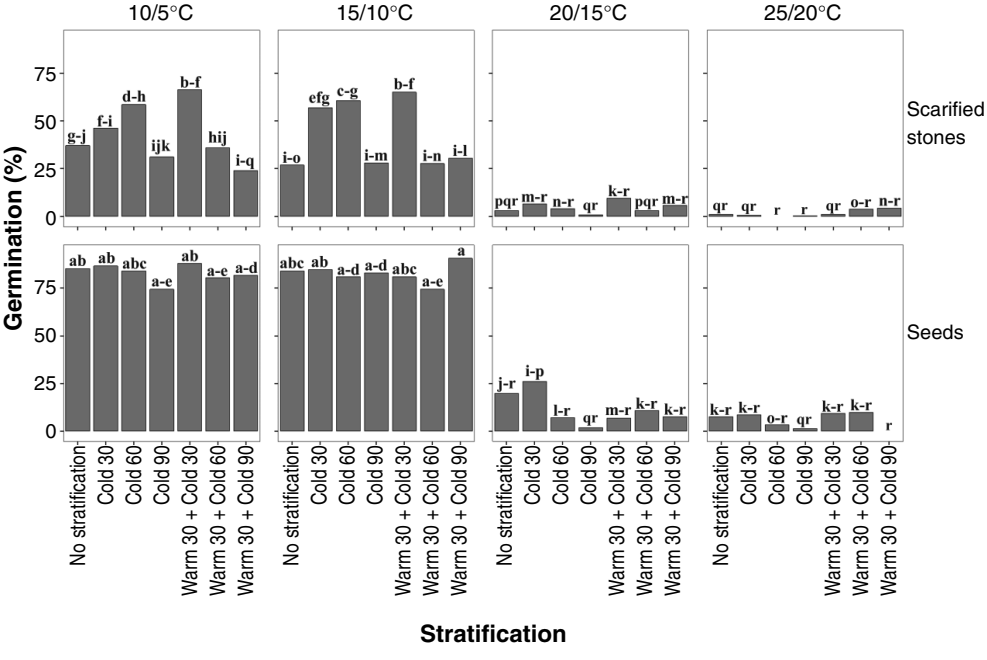

Figure 1. Germination of *Picconia azorica* seeds, after 35 weeks. Effect of temperature regime (10/5, 15/10, 20/15 and 25/20°C), type of stone treatment (acid scarification "Scarified stones" or removal the endocarp "Seeds") and stratification. Stratification treatments were: 30, 60 or 90 days cold (4°C); 30 days warm (20°C) + 30, 60 or 90 days cold (4°C). Results of Tukey test applied after ANOVA. Different letters indicate significant differences (Tukey HSD test, $\alpha = 0.05$). Seeds that germinated during the stratification treatment are not included.

No stratification or the 30 days cold and 30 days warm + 30 days cold stratification regimes resulted in the fastest germination (T_{50} 3.0 – 5.8 weeks). The remaining treatments resulted in slower germination with much higher T_{50} values (figure 2). For scarified stones, the 30 days warm + 30 days cold stratification treatment produced the fastest rate of germination (T_{50} = 20 weeks) but it was still less effective than using seeds (figure 3). Non-germinated seeds at the end of the stratification treatments were mostly decayed. An increase in size of the embryonic cotyledon leaves was only observed for cold treatments.

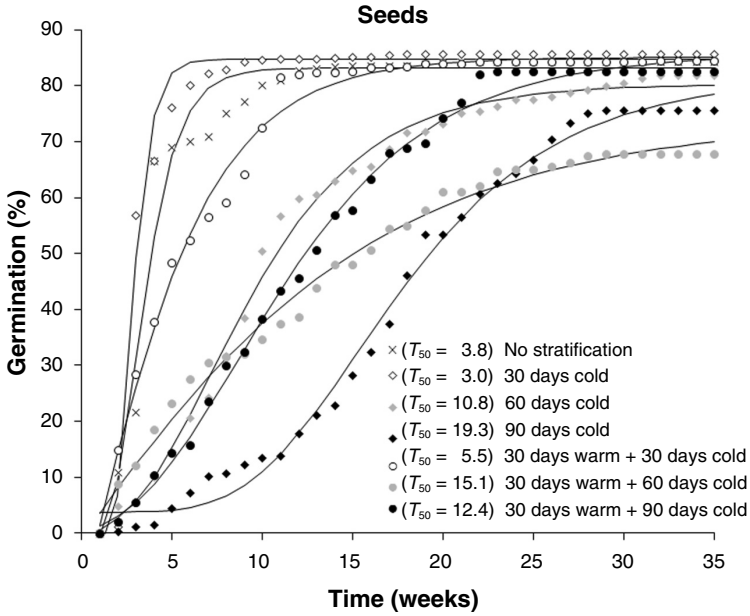

Figure 2. Germination of *Picconia azorica* seeds (endocarp removed). Accumulated seed germination curves for different stratification treatments, calculated globally for the two best temperature regimes (10/5 and 15/10°C). Observed (symbols) and expected (lines) germination curves, and T_{50} values (time for 50% germination), calculated using Gompertz model. For all the curves, the value of R^2 was above 0.9.

There were significant effects of stratification regime (F = 77.8; P < 0.001) and type of stone treatment (F = 28.0; P < 0.001) and a significant interaction effect of these factors (F = 9.2; P < 0.001) on germination rate. The treatments showing the highest germination rates were 30 days warm + 60 days cold and 30 days warm + 90 days cold, with 45.5-47.4% germination of seeds and 5.9-32.9% germination of scarified stones (figure 4).

Seedling growth

ANOVA showed significant differences in radicle growth (F = 9.8; P < 0.0001) and seedling survival (F = 15.2; P = 0.001) depending on the temperature regime used after germination. Survival increased (23.3, 36.7, 52.2, and 75.6%) as temperature decreased (25/20, 20/15, 15/10, and 10/5°C, respectively). Tukey test (α = 0.05) showed that the radicle grew significantly more at 10/5 and 15/10°C (14.0 ± 1.67 mm; 14.9 ± 1.52 mm) than

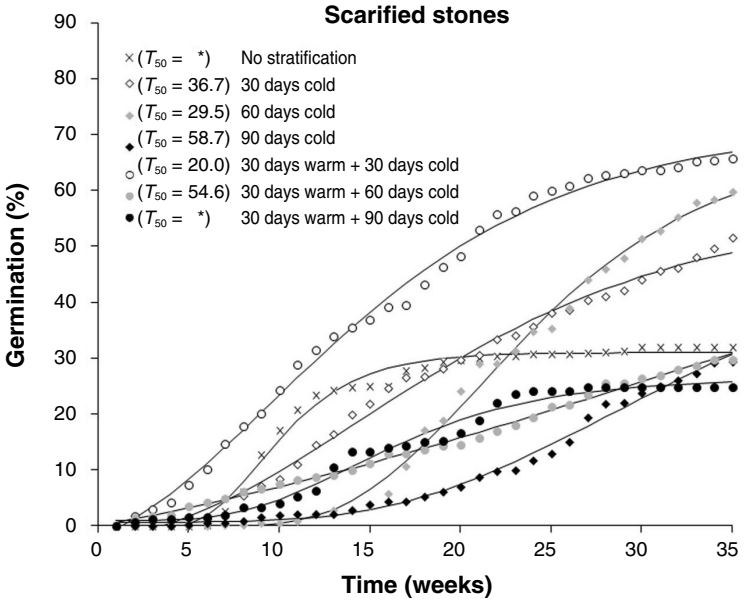

Figure 3. Germination of *Picconia azorica* acid scarified stones. Accumulated seed germination curves for different stratification treatments, calculated globally for the two best temperature regimes (10/5 and 15/10°C). Observed (symbols) and expected (lines) germination curves, and T_{50} values (time for 50% germination), calculated using Gompertz model. For all the curves, the value of R^2 was above 0.9.

Figure 4. Germination of *Picconia azorica* seeds (endocarp removed) or acid scarified stones during the stratification process [30, 60 or 90 days cold (4°C); 30 days warm (20°C) + 30, 60 or 90 days cold (4°C)]. Different letters indicate significant differences (Tukey HSD test, $\alpha = 0.05$).

at 20/15 and 25/20°C (3.6 ± 0.67 mm; 6.0 ± 1.50 mm). On average, germinated seeds took 103.3 ± 2.60 days at 10/5°C for the full development of the cotyledon leaves (uncovering the first true leaf pair), with an epicotyl length of 2-5 mm; higher temperatures were detrimental, resulting in no development with mortality rates of close to 100%.

Discussion

The results obtained in this research showed that endocarp removal resulted in higher germination than acid scarification which demanded an additional stratification treatment. Stony endocarps are common in Oleacea (Hill, 1933, 1937; Nikolaeva, 1969) and there is a large amount of evidence regarding the importance of both procedures in dormancy break (Legesse, 1993; Vachkoo *et al.,* 1993; Bandino *et al.,* 1999; Rostami and Shasavar, 2009). Similar results were reported by Ghayyad *et al.* (2010) for *Prunus mahaleb* and for *P. azorica* by Moreira *et al.* (unpublished). According to Baskin and Baskin (2004), a covering layer (or layers) can restrain embryo growth due to low growth potential of the embryo, and may thus be a component of physical dormancy. However, the embryos of *P. azorica* did not occupy the full length of the seeds at the time of imbibition, thus it does not seem plausible that the endocarp is acting as a mechanical barrier to their development. The results obtained in the imbibition test also ruled out the possibility of physical dormancy related to the presence of a water-impermeable layer. Water was absorbed by the endocarp and the seed, and it is probable that the gap that exists between the testa and the endocarp filled up with water, explaining the quick initial weight increase of stones.

In some species dormancy may be connected to the production of chemical inhibitors by the seed (Baskin and Baskin, 1998), including phenolic compounds present in the seed coat (Kong *et al.,* 2008). These compounds might accumulate in the endocarp cavity after imbibition and are leached out only when the endocarp is removed, allowing germination to proceed (Soltani, 2003). In fact, chemical analyses of *P. azorica* fruits indicated that phenolic compounds are mostly present in the seed coat. However, a preliminary investigation of their chemical composition did not detect the presence of common inhibitors such as those cited by Kong *et al.* (2008), suggesting the presence of novel compounds. In *P. azorica*, it is possible that these chemicals are leached out as the endocarp naturally decays due to microorganism activity and the extremely wet climate found in the Azores, which might have been replaced in our assays, by endocarp removal. Concluding evidence of the role of chemical inhibitors in *P. azorica* seed dormancy needs more specific testing. Nevertheless, the identification of the phenolic compounds conducted in this study may be helpful to delineate future research in order to better understand their effects in the germination of *P. azorica* seeds.

In *P. azorica,* stratification increased germination rate and velocity only for acid scarified stones, and exclusively in treatments corresponding to a maximum of 60 days (either cold or warm followed by cold). In fact, as also found for seeds, stratification for more than 30 days (cold) or 60 days (warm followed by cold) seems to be unfavourable for embryo development. Although the occurrence of some germination during stratification

using warm followed by cold might indicate a possible influence of the warm period, considering the literature available for the Oleaceae (Steinbauer, 1937; Piotto *et al.*, 2003; Bonner, 2001; Tilki and Cicek, 2005), and the number of seeds available, we opted to not use more than 30 days warm treatment in this experimental design. In future research, it will be interesting to further test the effect of warm stratification in embryo development. In general, the length of the stratification period required by *P. azorica* scarified stones to efficiently germinate was less than that reported for other Oleaceae (i.e. *Fraxinus* spp; Ellis *et al.*, 1985; Bonner, 2001). The tetrazolium test indicated initial viability values of ca. 100%, for the seeds used in this study thus it is probable that non-germinated seeds which did not appear necrotic at the end of the experiments were still dormant.

In this research, no significant effect of the addition of gibberellic acid was found on germination. The effect of the addition of growth regulators in the germination of *Oleaceae* seeds is not homogeneous; in some species there is an increase in germination (Sondheimer and Galson, 1966; Ashley, 2000; Hussain and Hussain, 2004) while in others, no effect was found (Villiers and Wareing, 1964; Arrillaga *et al.,* 1992).

In temperate climates in which water is not seasonally restricted, as is the case of the Azores Archipelago, temperature has been identified as one of the main factors governing changes in degree of dormancy (Benech-Arnold *et al.,* 2000). In the present study, alternating low temperatures between 5 and 15°C in conjunction with acid scarification of stones or endocarp removal induced the dormancy break of *P. azorica* seeds. The average temperatures for the coastal location under study (Azevedo, 1996) oscillate between 13-19°C in spring, 18-24°C in summer, 14-19°C in autumn, and 11-16°C in winter. Although the temperature regime between 10-15°C best corresponds to coastal winter temperatures, it is noteworthy that even lower temperatures also promoted dormancy break in the Azorean *Picconia*. This is to be expected, since average temperature decreases with increasing altitude, and the distribution range of *P. azorica* also encompasses locations at up to 700 m above sea level. This pattern of higher germination at lower temperatures is common to other woody endemic species of the Azores (Moura and Silva, 2010; Moreira *et al.,* unpublished) and might be the result of adaptation to the local climate.

According to our results, seedling growth (radicle extension and cotyledon leaf development) also required low temperatures. During seedling development at the most favourable temperature, cotyledon leaves remained enclosed by the tegument and were not fully developed until the endosperm was totally consumed, three months later. Development of the epicotyl occurred during this long process and when cotyledon leaves were released from the tegument, the first true leaf pair became visible. No development was observed at the warmest temperature and seedlings eventually became necrotic. This indicates an epicotyl dormancy which has also been reported for *Viburnum treleasei* Gand., another Azorean woody species (Moura and Silva, 2010). In general, low temperatures seem to be favourable to the germination of *P. azorica* seeds similarly to what was also was found for *V. treleasei* (Moura and Silva, 2010). Germination during stratification mainly occurred in treatments which combined warm and cold, which suggests that a warm temperature contributes to embryo development. Identically, in a study with *V. odoratissimum* Ker Gawl. and *V. tinus* L. seeds (Baskin *et al.*, 2008; Karlsson *et. al*, 2005) the authors found that embryos grew at higher temperatures. This led Baskin *et.*

al. (2008) to consider the existence of a simple morphophysiological dormancy (MPD) in *V. odoratissimum*. MPD is found in a number of plant families and according to Baskin and Baskin (1998, 2004), occurs in seeds with underdeveloped embryos, which also have a physiological dormancy. In *P. azorica*, germinated seeds, did not require cold stratification for radicle and epicotyl elongation, however within the temperatures tested, the lowest (10/5°C), was the most favourable. Cold stratification was also not necessary for the occurrence of radicle and epicotyl elongation in *V. odoratissimum* and *V, tinus,* although the optimal temperatures for these species were higher than those registered for *P. azorica.* Thus, seed characteristics and germination requirements in *P. azorica* suggest the occurrence of a non-deep simple epicotyl MPD in the sense described by Baskin *et al*. (2008), possibly also including the occurrence of a chemical inhibition by organic compounds mainly present in the seed coat.

Finally, it should be stressed that this research established a germination protocol which largely improved the rate and speed of the germination process. Although other methods have already been established, namely vegetative propagation, this research clearly showed that seed propagation of *P. azorica* is a feasible and viable method for obtaining good quality seedlings. This approach can be effectively used for the restoration of depauperate populations, while preserving their genetic diversity, which assumes particular importance in those islands where the number of individuals/populations is very low.

Concluding remarks

In summary, the best germination results were observed for non-stratified *P. azorica* seeds; scarified stones needed stratification for dormancy break. There was no effect of growth regulator addition. High temperature regimes had a negative effect on germination. Embryo structure and germination requirements seem to indicate the presence of a morphophysiological, epicotyl dormancy. To break seed dormancy and efficiently propagate *P. azorica*, we recommend the complete removal of the endocarp and germination of the seeds at 10/5 or 15/10°C with a 12-hour light period, without the addition of GA_3.

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References

Association of Official Seed Analysts AOSA (2000). *Tetrazolium Testing Handbook*, (*Oleaceae Revision 2006*). (ed. J. Peters), Association of Official Seed Analysts, www.aosaseed.com/TZwebsite/2006pdf/2006TZOleaceae.pdf

Arrillaga, I., Marzo, T. and Segura, J. (1992). Embryo culture of *Fraxinus ornus* and *Sorbus domestica* removes seed dormancy. *Hortscience,* **27**, 371.

Ashley, J.A. (2000). *The Effects of Seed Treatments on Germination of Dormant Fraxinus americana L. and Fraxinus pennsylvanica Marsh*. Seeds. *Honors Theses.* Paper 86. http://opensiuc.lib.siu.edu/uhp_theses/86

Azevedo, E.B. (1996). CIELO: Clima insular à escala global. [Insular climate on a global scale]. Universidade dos Açores, Angra do Heroísmo. 17 June 2009. http://www.climaat.angra.uac.pt/cielo

Bandino, G., Sedda, P. and Mulas, M. (1999). Germination of Olive Seeds as Affected by Chemical Scarification, Hot Water Dip and Gibberellic Acid Treatments. *Acta Horticulturae,* **474**, 35-38.

Baskin, C.C. and Baskin, J.M. (1998). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination,* p. 666, Academic Press, San Diego.

Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy. *Seed Science Research,* **14**, 1-16.

Baskin, C.C. and Baskin, J.M. (2005). Seed dormancy in trees of climax tropical vegetation types. *Tropical Ecology,* **46**, 17-28.

Baskin, C.C., Chien, C.T., Chen, S.Y. and Baskin, J.M. (2008). Germination of *Viburnum odoratissimum* seeds: A new level of morphophysiological dormancy. *Seed Science Research*, **18**,179-184.

Benech-Arnold, R.L., Sânchez, R.A., Forcella, F., Kruk, B.C. and Ghersa, C.M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Research,* **67**, 10-122.

Bewley, J.D. and Black, M. (1994). *Seeds. Physiology of Development and Germination* (2nd edition). Plenum Press, New York.

Bonner, F.T. (2001). *Fraxinus L.* In: Bonner F, Krugman S, (Tech. coord.). *Woody plant seed manual*. U.S. Dept. Agr. Forest Serv., Washington, D.C. 6 May 2006. pp 1-14. http://www.nsl.fs.fed.us/wpsm/Fraxinus.pdf (29 May 2011).

Cardoso, P., Borges, P.A.V., Costa, A. C., Cunha, R. T., Gabriel, R., Martins, A.M.F., Silva, L., Homem, N., Martins, M., Rodrigues, P., Martins, B. and Mendonça, E. (2008). A perspectiva arquipelágica - Açores. [The archipelagic prospective - Azores]. In *TOP 100 - As Cem Espécies Ameaçadas Prioritárias em Termos de Gestão na Região Europeia Biogeográfica da Macaronésia [Top 100 – the One Hundread Priority Threatned Species for Management in the European Macaronesian Biogeographic Region],* (eds. J.L. Martín, M. Arechavaleta, P.A.V. Borges and B. Faria), pp. 421-449, Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias.

Crisosto, C. and Sutter, E.C. (1985). Role of the endocarp in "Manzanillo" olive seed germination. *Journal of American Society for Horticultural Science,* **110**, 50-52.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1985). *Handbook of Seed Technology for Genebanks Vol II: Compendium of Specific Germination Information and Test Recommendations*. *Handbooks for Genebanks Nº 3,* International Board for Plant Genetic Resources, Rome.

Fabbri, A., Bartolini, G., Lambardi, M. and Kailis, S. (2004). *Olive Propagation Manual*, p. 152. CSIRO Publishing, Collingwood, Australia.

Fagundo, M. and Isidoro, A. (2004). *Propagação das Espécies Lenhosas Endémicas dos Açores.* [*Propagation of Azorean Endemic Woody Species*], p. 47. Direcção Regional dos Recursos Florestais. Serviço Florestal do Nordeste.

Ferreira, R.C., Piredda, R., Francesca Bagnoli, F., Bellarosa, R., Attimonelli, M., Fineschi, S., Schirone, B. and Simeone, M.C. (2010). Phylogeography and conservation perspectives of an endangered macaronesian endemic: *Picconia azorica* (Tutin) Knobl. (Oleaceae). *European Journal of Forest Research,* **130**, 181-195.

Ghayyad, M., Kurbysa, M. and Napolsy, G. (2010). Effect of Endocarp Removal, Gibberelline, Stratification and Sulfuric Acid on Germination of Mahaleb (*Prunus mahaleb* L.) Seeds. *American-Eurasian Journal of Agricultural and Environmental Science,* **9**, 163-168.

Gompertz, B. (1825). On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical Transactions of the Royal Society,* **115**, 513-585.

Hill, A.W. (1933). The method of germination of seeds enclosed in a stony endocarp. *Annals of Botany,* **47**, 873-887.

Hill, A.W. (1937). The method of germination of seeds enclosed in a stony endocarp. II. *Annals of Botany,* **51**, 239-256.

Hussain, A.K.H. and Hussain, A.M.S. (2004). Influence of the gibberelic acid on the germination of the seeds of olive-tree *Olea europea* L. *Journal of Central European Agriculture,* **5**, 1-4.

Karlsson, L.M., Hidayati, S.N., Walck, J.L. and Milberg, P. (2005). Complex combination of seed dormancy and seedling development determine emergence of *Viburnum tinus* (Caprifoliaceae). *Annals of Botany*, **95**, 323-330.

Kong, L., Wang, F., Si, J., Feng, B. and Li, S. (2008). Water-soluble phenolic compounds in the coat control germination and peroxidase reactivation in *Triticum aestivum* seeds. *Plant Growth Regulation,* **56**, 275-283.

Laterra, P. and Bazzalo, M.E. (1999). Seed-to-seed allelopathic effects between two invaders of burned pampa grasslands. *Weed Research,* **39**, 297-308.

Legesse, N. (1993). Investigations on germination behaviour of wild olive seeds and the nursery establishment of the germinants. *Sinet: an Ethiopian Journal of Science,* **16**, 71-81.

Maciel, G.B. (1995). Influência do tempo de conservação das sementes na germinação de espécies vasculares endémicas dos Açores. [Influence of conservation time in the germination of Azorean vascular endemic species]. *Boletim da Sociedade Broteriana,* **67**, 171-186.

Maciel, G.B. (1996). Influência da temperatura e da luz na germinação de espécies vasculares endémicas dos Açores. [Influence of temperature and light in the germination of Azorean vascular endemic species]. *Açoreana,* **8**, 209-218.

Martin, A.C. (1946). The comparative internal morphology of seeds. *American Midland Naturalist*, **36**, 513-660

Martins, J., Moreira, O., Silva, L. and Moura, M. (2011). Propagation of the endangered Azorean endemic tree, *Picconia azorica*, by air layering. *Archipelago, Life and Marine Sciences,* **28**, 39-46.

Moura, M. and Silva, L. (2010). Seed germination of *Viburnum treleasei* Gand., an Azorean endemic with high ornamental potential. *Propagation of Ornamental Plants,* **10**, 129-135.

Nikolaeva, M.G. (1969). *Physiology of deep dormancy in seeds.* Izdatel'stvo "Nauka," Leningrad. [Translated from Russian by Z. Shaprio, National Science Foundation, Washington, DC].

Piotto, B., Bartolini ,G., Bussotti, F., Calderón García, A.A., Chessa, I., Ciccarese, C., Ciccarese, L., Crosti, R., Cullum, F.J., Di Noi, A., García-Fayos, P., Lambardi, M., Lisci, M., Lucci, S., Melini, S., Muñoz Reinoso, J.C, Murranca, S., Nieddu, G., Pacini, E., Pagni, G., Patumi, M., Pérez García, F., Piccini, C., Rossetto, M., Tranne, G., Tylkowski, T. (2003). Fact Sheets on the Propagation of Mediterranean Trees and Shrubs from Seed. In *Seed Propagation of Mediterranean Trees and Shrubs,* (eds. B. Piotto and A. Di Noi), pp. 11-51, APAT – Agency for the Protection of the Environment and for Technical Services, Rome.

Rainha N., Lima E., Baptista J. and Rodrigues, C. (2011). Antioxidant properties, total phenolic, total carotenoid and chlorophyll content of anatomical parts of Hypericum foliosum. *Journal of Medicinal Plants Research,* **5**, 1930-1940.

Rostami, A.A. and Shasavar, A. (2009). Effects of Seed Scarification on Seed Germination and Early Growth of Olive Seedlings. *Journal of Biological Sciences,* **9**, 825-828.

Silva, L., Martins, M., Moura, M. and Maciel, G.B. (2009). *Azorean Vascular Flora: Priorities in Conservation,* 122 pp. CCPA and Amigos dos Açores.

Silva, L., Moura, M., Schaefer, H., Rumsey, F. and Dias, E.F. (2010). List of Vascular Plants (Tracheobionta). In *A List of the Terrestrial and Marine Biota from the Azores* (eds. P.A.V. Borges, A. Costa, R. Cunha, R. Gabriel, V. Gonçalves, A.F. Martins, I. Melo, M. Parente, P. Raposeiro, P. Rodrigues, R.S. Santos, L. Silva, P. Vieira and V. Vieira), pp. 117-163, Princípia, Cascais.

Soltani, A. (2003). Improvement of seed germination of *Fagus orientalis* Lipsky. Acta Universitatis Agriculturae Sueciae. *Silvestria,* 275. Sondheimer, E. and Galson, E.C. (1966). Effects of abscisin II and other plant growth substances on germination of seeds with stratification requirements. *Plant Physiology*, **41**, 1397-1398.

Sotomayor-Leòn, E.M. and Duran-Altisent, J.M. (1994). Breaking of dormancy in olive (*Olea Europaea* L.) seeds. *Acta Horticulturae,* **356**, 137-142.

Steinbauer, G.P. (1937). Dormancy and germination of *Fraxinus seeds. Plant Physiology,* **12,** 813-824.

Tilki, F. and Cicek, E. (2005). Effects of stratification, temperature and storage on germination in three provenances of *Fraxinus angustifolia subsp. oxycarpa seeds. Turkish Journal of Agriculture and Forestry,* **29**, 323-330.

Vachkoo, A.M., Mughal, M.S., Gupta, O.P. and Sharma, K.C. (1993). Effects of some chemical and mechanical treatments on seed germination of olive (*Olea cuspidate* Wall.). *Advances in Horticulture and Forestry,* **3**, 105-108.

Villiers, T.A. and Wareing, P.F. (1964). Dormancy in fruits of *Fraxinus excelsior* L. *Journal of Experimental Botany,* **15**, 359-367.

Voyiatzis, D.G. (1995). Dormancy and germination of olive embryos as affected by temperature. *Physiologia Plantarum,* **95**, 444-448.

Voyiatzis, D.G. and Porlingis, I.C. (1987). Temperature requirements for the germination of olive seeds (*Olea europaea* L.). *Journal of Horticultural Science,* **62**, 405-411.

Zar, J.H. (1999). *Biostatistical Analysis* (4th edition). Prentice Hall, New Jersey.