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Breaking seed dormancy of three orthodox Mediterranean *Rosaceae* species

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Abstract

Biodiversity levels could be enhanced when regenerating a site by seed-derived seedlings. However, seed dormancy poses limitations for many species. As a result, nurseries either produce seedlings from species where dormancy is not an obstacle, or they propagate through cuttings with the risk of decreasing the genetic diversity within and among species at the regenerated sites. In the present study, breaking of seed dormancy was investigated in valuable Mediterranean species of *Prunus avium*, *Prunus spinosa* and *Rosa canina* Specifically, in order to break dormancy, seeds of those species were warm-, cold- stratified and chemically treated. Based on the results, maximum germination for *P. avium* was 12% when seeds were warm stratified for four weeks altered with eight weeks of cold stratification. For *P. spinosa*, maximum percent germination was 26% when seeds were warm stratified for two weeks and continuously altered for eight weeks of cold stratification. Finally, for *R. canina* maximum percent germination was 40% under four weeks of warm stratification altered with twenty weeks of cold stratification, when seeds were pretreated with H_2SO_4 for 15 min. A maximum of twelve weeks of cold stratification for *P. avium*, *P. spinosa* and 20 weeks for *R. canina* provided almost zero percent germination. The results indicated that all three species experienced intense dormancy levels suggesting that those species need to be treated properly prior to sowing. Nonetheless, additional experiments are needed to achieve greater germination percentage of highly valuable species in order to encourage seed derived seedling production.

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Introduction

When regenerating or reforesting a site the ideal planting scenario for increasing genetic diversity is either by direct seeding or by seed-derived seedlings. However, seed dormancy poses limitations in production of many species. As a result, nurseries either focus on producing seedlings from species where dormancy is not an obstacle, or they propagate seedlings through cuttings. That ultimately decreases the genetic diversity in terms of complexity within and among species at the regenerated sites.

Prunus avium, *Prunus spinosa* and *Rosa canina* in addition to their remarkable beauty, are highly valuable species based on their ecological and economic input. They provide food and shelter to wildlife, while humans also use them for their own benefits. Specifically, the extracted oil from *R. canina* is used for aromatherapy, while it is also used as a drink due to its therapeutic properties in relation to its good taste (Dogan and Kazankaya, 2006; Ercisli, 2007; Kazaz *et al.*, 2010). Further, its flower is undoubtedly one of the most aesthetically pleasing remarks and one of the additional reasons why it is highly cultivated by humans. The flower of both *P. avium* and *P. spinosa* are also very aesthetically pleasing in combination to their high contribution to wildlife, mainly due to their edible fruits. Moreover, the wood quality of *P. avium* is very valuable for making furniture.

However, the seeds of these species experience deep dormancy levels at dispersal time (Baskin and Baskin, 1998; Jensen, Eriksen 2001; Finch-Savage *et al.* 2002; Meyer, 2008). The intensity of seed dormancy might vary from year to year, most influencing factors being temperature fluctuation and genetics. So, despite the benefits that are gained when regenerate sites from seed derived seedlings, the orthodox nature of those seeds pose limitation to their production (Baskin and Baskin, 1998; Hosafcı *et al.*, 2005; Alp *et al.*, 2009; Werlemark, 2009).

For *Prunus* spp., in particular *P. avium*, it has been noted that alternated stratification of warm and cold temperatures have also affected positively the breaking of seed dormancy (Werlemark *et al.*, 1995; Chen *et al.*, 2007; Eşen *et al.*, 2009; Zhou *et al.*, 2009). For *R. canina* it has been reported that the germination percent during the first year was ranging from zero to ten that reached 18% during the second year after seeds were been treated with warm and cold stratification (Alp *et al.*, 2009). Further, it has been noted that high temperatures as well as intense light have positive affect on the percent seed germination in *Rosa* spp. when compared to low temperature and low light conditions (Werlemark *et al.*, 1995). It has also been shown that acid treatments as well as high temperatures have weakened the hard pericarp of *R. canina* that stimulated germination (Werlemark *et al.*, 1995).

In the present study an attempt was made to investigate the possibilities of breaking seed dormancy of *P. avium, P. spinosa* and *R. canina,* the three valuable Mediterranean species distributed in Greece. Specifically, experiments of warm altered with cold stratification were studied. For *R. canina*, in addition to the above treatments, seeds were also treated chemically to limit germination inhibition due to hard pericarp. It was hypothesized that all three species experience dormancy levels, with *R. canina* requiring extra pericarp treatment.

Materials and Methods

Seeds of all studied species that belonged to *Rosaceae* family were collected from wild varieties of Greek provenances, during autumn 2010. Specifically, for *Prunus avium* the seeds were collected from Vermio, for *Prunus spinosa* L. from Lachana and for *Rosa canina* from Xiloupoli. Collection was carried out from healthy, open crown plants with main seed selection criterion the ripening color of the fruits in order to guarantee proper seed maturation. Continuously, the seeds were separated from their fruiting part and were stored in polyethylene bags in a walking cooler (5±2 ºC) until initiation of the experiments that took place three months later.

Prior experiments, seeds of *P. avium* and *P. spinosa* were soaked in water for 24 hrs. Continuously, they were placed in petridishes that retained wet sand. To meet the experimental needs, a number of seeds were placed in cooler (5 ± 2 ºC) to be cold stratified (C), with durations of two- (C2), four- (C4), six- (C6), eight- (C8) and ten- (C10) weeks. Further, a number of seeds were placed in growth chamber at 20 ºC (KB8000FL, Termaks AS, Bergen, Norway) to serve warm stratification conditions (W) with duration of two- (W2), four- (W4), six- (W6), eight- (W8) and ten- (W10) weeks. Part of the warm stratified seeds were continuously placed in the cooler to be cold stratified with durations of two- (C2), four- (C4), six- (C6), eight- (C8) and ten- (C10) weeks. Evaluation of seed germination was conducted every two weeks for each seed treatment by placing in the growth chamber a set of five petridishes, with each petridish retaining 20 seeds. The growth chamber (KB8000FL, Termaks AS, Bergen, Norway) was set at 14 hr day-light at 20°C altered with 10 hr of night-light at 15°C temperature (250 µmol m^{-2} s^{-1} photosynthetic photon flux density) and 80±10% air relative humidity.

Similarly to *P. avium* and *P. spinosa*, a number of *R. canina* seeds were soaked in H_2O for 24 hrs. In addition, a number of seeds were also treated with H_2SO_4 for duration of five-, ten- and fifteen min and were soaked for 24 hrs in water. Continuously, part of those seeds were cold stratified by placing them in cooler (5°C± 2°C) to serve cold stratification conditions (C), with duration of four- (C4), eight- (C8), twelve- (C12), sixteen- (C16) and twenty- (C20) weeks. Another set of seeds was warm stratified with similar durations as that of cold stratification (W4, W8, W12, W16 and W20). In addition, a number of seeds were warm stratified and continuously were cold stratification (similarly to *P. avium* and *P. spinosa* seeds) to test for the combined effect of warm and cold stratification. Seed germination for *R. canina* species was evaluated every four weeks based on five petridishes that each retained 20 seeds each. The presented results are based on the mean values of those five petridish (replications) and their standard deviation for each studied treatment.

Results and Discussion

Based on the results, it has been indicated that seeds of all three Mediterranean species experienced seed dormancy. This implies that in order to successfully propagate these species by seeds to guarantee genetically diverse regenerating material, further treatment needs to take place. The treatment that seemed to work better for all three species was combination of warm and cold stratification.

Specifically, based on the timeframes of experiments, for *P. avium*, four weeks of warm stratification when altered with eight weeks of cold stratification seemed to work better (12%), while for *P. spinosa* combination of two weeks warm stratification altered with eight weeks cold was ideal combination (26%) (Fig. 1). For *R. canina* L. 15 min of H_2SO_4 pre-treatment followed by combination of four weeks of warm stratification altered with 20 weeks of cold gave highest germination percent (38%) (Fig. 2). In addition, for all studied species, cold as well as warm stratification treatments for maximum of 10 weeks for *P. avium* and *P. spinosa* and 20 weeks for *R. canina*, did not seem to positively affect germination (Fig. 1, Fig. 2). Only *P. spinosa* reached 10 % under four weeks of warm stratification.

For the majority of species, breaking of dormancy is triggered by environmental cues (Baskin and Baskin, 1998). In the case of *P. avium* and *P. spinosa* it seemed that there was an imitation of Mediterranean environments characterized by warm summers (warm stratification) and cold winters (cold stratification), which resulted in greater germination percentage of those species. For *Prunus* spp., in particular *P. avium*, it was noted that alternated warm and cold temperature positively affected in breaking seed dormancy (Werlemark *et al.*, 1995; Eşen *et al.*, 2009; Zhou *et al.*, 2009).

Although germination percentage of both *P. avium* and *P. spinosa* (12 and 26%, respectively) were fairly low, it is in agreement with the previous results (Finch-Savage *et al.*, 2002). Particularly for *P. avium*, difficulty in evaluating seed maturation at harvest time by fruit color and its substantial effect in the intensity of seed dormancy was pointed (Finch-Savage *et al.*, 2002). So, despite the fact that only well-ripen fruits were collected, some deviation in ripening color might have interfered with the experimental outcome. High germination percentages were also achieved by other researchers, but only after chemical seed treatments (Çerinbaş and Koyuncu, 2007).

For *R. canina* seeds treated with sulfuric acid followed by warm and cold stratification, gave promising results in breaking seed dormancy (Fig. 2). Specifically, seeds that were treated with 15 min of sulfuric acid were placed for four weeks under warm stratification that was altered with twenty weeks of cold stratification, gave 38 % germination. Under same warm (W4) and cold (C20) stratification conditions, seeds that were treated with 5 and 10 min sulfuric acid showed 10 and 30 % germination. Under warm stratification of eight weeks when combined with cold stratification of twenty weeks, seeds that were treated with sulfuric acid for 15 min were just provided with water showed highest percent germination (20%) followed by 15% germinated seeds treated with H_2SO_4 for 10 min. Further, warm stratification of twelve weeks when combined with sixteen weeks of cold stratification provided 16% germinated seeds after they were treated with sulfuric acid for 15 min, while those treated for 10 and 15 min showed 12 % germination.

For *R. canina* seeds, both morphology of seed pericarp as well as physiology of embryo posed limitations to seed germination. It has been indicated that hard stony pericarp of *Rosa* spp. did not allow any water or air diffusion, while radical

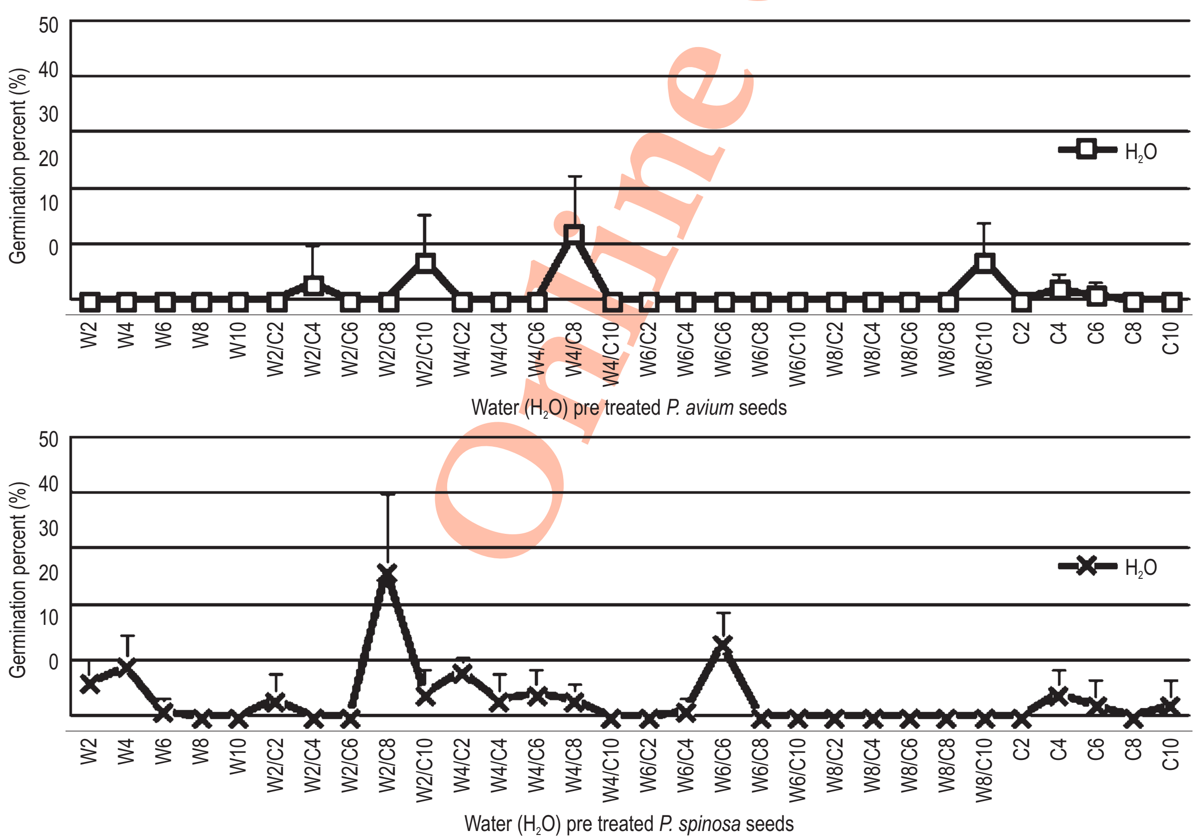

Fig. 1 : Germination percent of *Prunus avium* and *Prunus spinosa* when seeds were warm- and cold- stratified as well as combination of warm and cold stratification; SE of means are shown as vertical bars

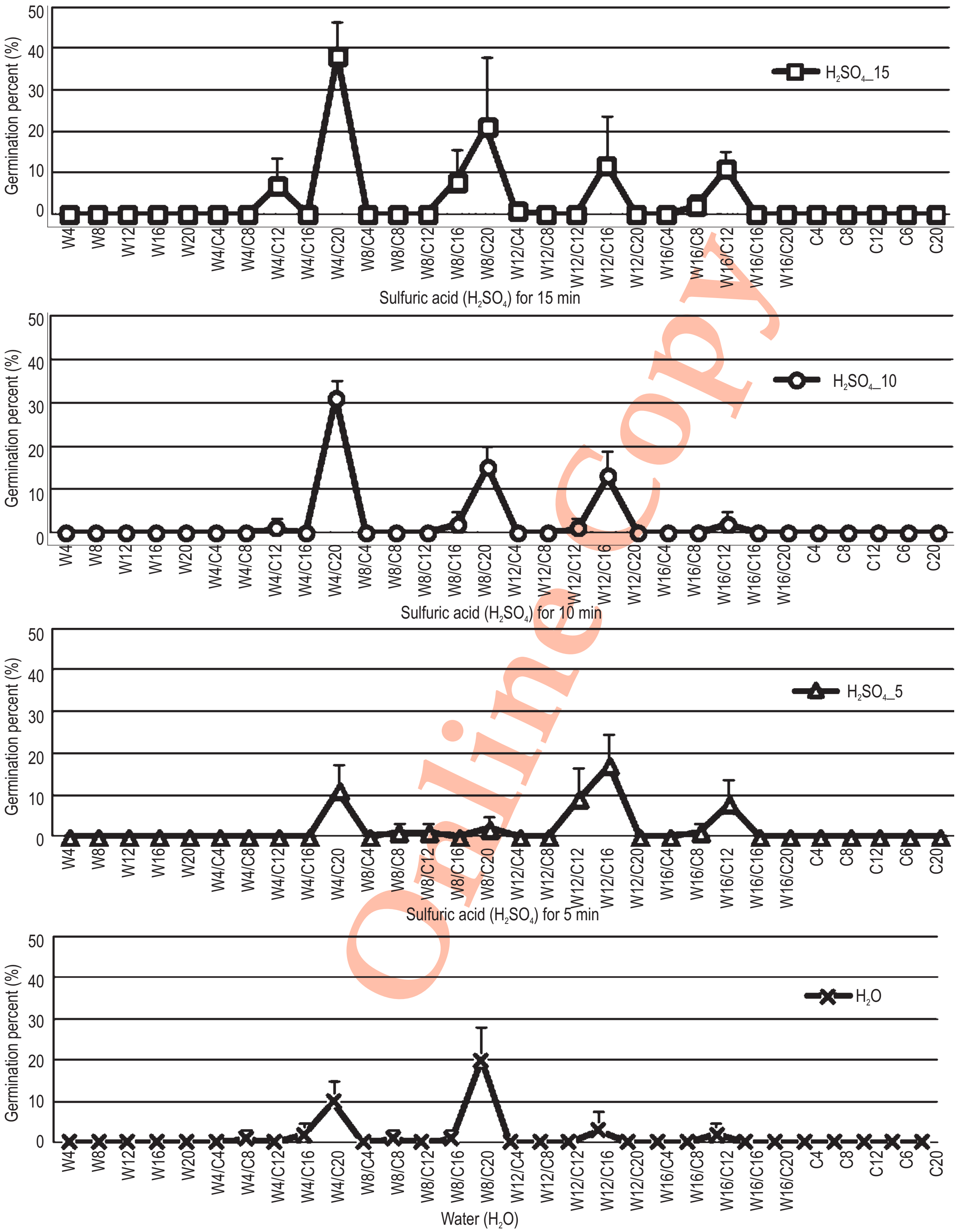

Fig. 2 : Germination percent of *Rosa canina* when seeds were pretreated with sulfuric acid for 5, 10 and 15 minutes and continuously were warm-, cold-stratified as well as combination of warm and cold stratification; standard errors of means are shown as vertical bars

protrusion was also inhibited (Ueda, 2003; Zlesak, 2007; Meyer, 2008). This was verified in our study since seeds that were treated with sulfuric acid provided positive germination results. Practically, it also suggests that if "pericarp" of *R. canina* is treated prior to any further experimentation it helps in increasing the percentage of germinated seeds.

Specifically, the best germination results (38 %) for *R. canina* was achieved on combining pericarp scarification (15 min of sulfuric acid) with warm (four weeks) and cold stratification (20 weeks). Alp *et al.* (2009) also found for *R. canina* that warm stratification of 10, 11 and 12 weeks followed by 20 weeks of cold stratification initiated seed germination upto 18 %. Similarly, Nadeem *et al.* (2013) also noted greater germination (18.5 %) for *Rosa* x *hybrid* seeds that were treated with 30 days of warm followed by 60 days of cold stratification and 30% H_2SO_4 for 10 min. This implies that *R. canina* seeds should treated in combination in order to achieved high germination rate. Specifically, sulfuric acid treatments when combined with altered warm and cold stratification could help nurseries increase the percentage of seed germination for those valuable species.

In the present study, 20 % germination was also noted for seeds that were warm and cold stratified (8 and 20 weeks, respectively), with no sulfuric acid treatment. This shows that a small percent of *R. canina* seeds managed to germinate despite stony pericarp, indicating the ability of species to adapt and germinate even when no scarification takes place under field conditions. However, it needs to be mentioned that greater germination percentages have been noted for *Rosa* spp. seeds that were treated with additional chemicals such as GA_3 (Kazaz *et al.*, 2010), in the present study.

The results of the present study is in agreement with previous studies indicating that these species experience deep dormancy levels (Baskin and Baskin, 1998; Hajian and Khosh-Khui, 2000; Finch-Savage *et al.*, 2002; Stephen *et al.*, 2003), posing a barrier in producing seed-derive seedlings. Therefore for successful propagation of these species by seeds in nurseries, further treatments should be given. The best way for this is combination of specific timeframes of warm and cold stratification for all 3 species, which could be used by the nurseries to successfully produce seed-derived seedlings. These seedlings will have potential to support the biodiversity levels at regenerating sites.

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