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# EFFECT OF SULPHURIC ACID SCARIFICATION, COLD MOIST STRATIFICATION AND GIBBERELLIC ACID ON GERMINATION OF *PALIURUS SPINA-CHRISTI* MILL. SEEDS

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## *Abstract*

*Paliurus spina-christi* seeds were subjected to several treatments in order to overcome dormancy and to maximize germination. Seeds were subjected to sulphuric acid scarification for 0, 30, 60 and 90 minutes and then were stratified at 3–5ºC for 0, 4, 8 and 12 weeks (1st experiment) or treated with 500, 1000 and 2000 ppm gibberellic acid (2nd experiment). In the first experiment, increasing scarification of non-stratified seeds from 30 to 60 or 90 minutes germination percentage increased significantly. Moreover, cold stratification treatments of non-scarified seeds resulted in very low germination percentages. The combination of acid scarification and cold stratification treatments improved significantly germination percentages. In the second experiment, treatment with gibberellic acid, regardless of concentration, of scarified seeds improved significantly germination percentages. The concentration of gibberellic acid was found to effect germination only in 30 minutes scarified seeds. In treated seeds with 500 or 1000 ppm gibberellic acid, a significant increase of germination percentage together with the increase of time of scarification from 30 to 60 or 90 minutes was observed. The results revealed that scarification was more effective than cold stratification in improving seed germination when treatments were applied alone. The highest germination percentages were observed when acid scarification was followed by cold stratification or treatment with gibberellic acid.

**Key words:** cold stratification, gibberellic acid, *Paliurus spina-christi*, seed germination, sulphuric acid scarification.

## Introduction

*Paliurus spina-christi* Mill (Christ's thorn) is a very thorny shrub up to 4 m tall native to South Eastern Europe and West Asia. In Greece it is common in the continental part of the country and on some, more northern islands (Boratynski et al. 1992). This species is one of the most important components of overgrazed and eroded ar-

eas in Greece. The ability to withstand in dry and degraded sites makes it suitable for restoration of disturbed areas.

Regeneration from seeds is the most often used and cheapest method of propagation in many forest species. But, this propagation technique exhibits difficulty due to seed dormancy. The causes of seed dormancy can be attributed to exogenous (seed coat and other structures prevent germination) and endogenous (embryo characteristics that prevent germination) factors (Nikolaeva 1977). Scarification or stratification treatments are used to overcome seed dormancy. Scarification refers to any procedure that modifies the seed coat to permit the entry of water and the exchange of gasses so that the germination process begins. Various methods of scarification are used to break dormancy (chemical, mechanical, hot water) (Bonner et al. 1994). Moist stratification (cold or warm) is widely used for breaking embryo dormancy and enhancing the germination of seeds in numerous species (Macdonald 1993). Various solutions of hormones are used to accelerate breaking seed dormancy. Gibberellic acid (GA) is one of the hormones that can be used to partially or fully replace the necessary period of cold moist stratification (Baskin and Baskin 1998).

There are conflicting results regarding to the contribution of acid scarification and moist cold stratification in overcoming *P. spina-christi* seed dormancy. Takos et al. (2001) and Piotto et al. (2003) attributed *P. spina-christi* seed dormancy to hard seed coat and recommended sulphuric acid scarification or cold moist stratification for breaking seed dormancy. In contrast, Olmez et al. (2007) reported that treatment of seeds with sulphuric acid gave high germination percentages whereas cold stratification treatment resulted in very low germination percentages.

The assumption that both treatments overcome seed dormancy and enhance germination incurs the risk of poor germination due to inadequate treatment. Furthermore, there is a lack of published literature describing the effects of gibberellic acid on seed germination.

The purpose of this research was to: i) examine the effectiveness of acid scarification, cold moist stratification and gibberellic acid ($GA_3$) treatments on *P. spina-christi* seed germination; ii) describe the effects of acid scarification and cold stratification treatment combinations on germination; iii) describe the effects of acid scarification and gibberellic acid ($GA_3$) treatment combinations on germination; iv) propose treatments that maximize germination of *P. spina-christi* seeds.

## Materials and Methods

Mature fruits (drupes) of *P. spina-christi* were collected in November 2008 from a number of plants (more than 10) in NE Greece (41º07´41´´N, 25º14´11´´E, 40 m elevation). The fruits were spread upon the floor and were then crushed by a heavy cylinder in order to break the hard exocarp and extract the seeds. Sieving and flotation were used to remove trash (parts of fruits) and also empty and broken seeds. Then, the clean seeds were spread on filter paper and left to dry. After drying, the seeds were stored in glass containers in the refrigerator (3–5ºC) until the beginning of the experiments.

**Seed treatment**

Two experiments were conducted in order to determine the effects of acid scarification, cold moist stratification and gibberellic acid ($GA_3$) treatments on *P. spina-christi* seed germination. Seeds were immersed in concentrated (95–97%) sulphuric acid for 0, 30, 60 and 90 min. After treatment the seeds were removed from the acid and were washed thoroughly under running water. Acid scarified and non-scarified seeds were subjected to the following treatments. A certain amount of the above seeds were mixed with wet sterilized river sand and were placed in plastic containers and underwent cold stratification at 3–5ºC for 4, 8 and 12 weeks (first experiment). Each container corresponded to a different duration of immersion in sulphuric acid (there were 4 plastic containers). In total, 12 treatments (combinations between acid scarification and cold stratification) were applied. Another amount of seeds were treated with gibberellic acid ($GA_3$) (second experiment). 120 seeds of each treatment with sulphuric acid (0, 30, 60 and 90 min) were soaked in 500, 1000 or 2000 ppm $GA_3$ for 24 hours. In total, 12 treatments (combinations between acid scarification and $GA_3$) were applied. Moreover, acid scarified (30, 60 and 90 min) and non-scarified seeds (0 min) were subjected immediately to a germination test without cold stratification (0 weeks) or $GA_3$ treatment (0 ppm).

**Germination test**

The germination experiment was conducted in the laboratory of Silviculture, in the Faculty of Forestry and Natural Environment of Aristotle University of Thessaloniki. In the first experiment, at the end of each stratification period a random sample of 120 seeds was taken out from each plastic container and randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). In the second experiment after the treatment with $GA_3$ the seeds (120 seeds per acid scarification duration and $GA_3$ concentration) were randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). In both experiments, for each treatment there were 4 replications of 30 seeds. Sterilized river sand moistened with distilled water was used as substrate in the plastic Petri dishes. Prior to the arrangement of seeds in Petri dishes they were dusted with fungicide (Captan) to avoid fungi development. The Petri dishes were randomly arranged on the shelves of the growth chamber.

The temperature in the growth chamber was set at 20ºC for a 16 h dark period and 25ºC for an 8 h light period. Seed germination was defined as the appearance of a radicle, at least 2 mm long, according to the rules of the International Seed Testing Association (1999). Germinated seeds were counted each week for 6 weeks. Finally, the germination percentage was calculated for each replication. The germination percentage of each treatment was calculated from the average of the 4 replication percentages.

**Statistical analysis**

In both experiments the experimental design was a completely randomised factorial design. In the first experiment the factors were the duration of acid scarification and the length of cold stratifica-

tion period (4 x 4 factorial design). In the second experiment the factors were the duration of acid scarification and the concentration of gibberellic acid (4 x 4 factorial design). The data was analysed using the ANOVA method in the frame of the GLM (General Linear Model). The germination percentage data was transformed to arc-sine square root values, before analysis (Snedecor and Cochran 1980). The transformed data was checked for normality and homogeneity of variances and was then analysed by ANOVA, while the comparisons of the means were wade using the Bonferroni test (Klockars and Sax 1986).

Finally, the Duncan test was used to detect significant differences among the best 10 treatment combinations (in total 28) with a probability of 95% ($a = 0.05$) (Klockars and Sax 1986).

All statistical analyses were carried out using SPSS 12.0 (SPSS, Inc., USA).

## Results

### Experiment 1

The analyses of variance indicated that there were significant differences on germination percentages at $a = 0.05$ affected by: (a) acid scarification [$F(3,48) = 600.808$, $P = 0.000$], (b) cold stratification [$F(3,48) = 93.304$, $P = 0.000$] and (c) the interaction between the factors acid scarification and cold stratification [$F(9,48) = 29.032$, $P = 0.000$]. The main effects of two factors are not analyzed since there is significant interaction between them. For seeds that were not stratified, 60 or 90 min scarified seeds gave higher germination percentages than non-scarified, and those that were scarified for 30 min ($p < 0.05$) (Table 1). In each of the 3 periods (4, 8 and 12 weeks) of stratifications, seeds scarified for 30, 60 and 90 min exhibited higher germination percentages than non-scarified ($p < 0.05$), while no significant differences were observed among them ($p > 0.05$). Non-scarified seeds that were subjected to stratification for 0, 4, 8 and 12 weeks exhibited very low germination percentages without statistically significant differences among them ($p > 0.05$). In each of the 3 treatments (30, 60 and 90 min) with sulphuric acid, seeds stratified for 4, 8 and 12 weeks exhibited higher germination percentages than non-stratified ($p < 0.05$), while no significant differences were observed among them ($p > 0.05$) (Table 1).

### Experiment 2

The analyses of variance indicated that there were significant differences on germination percentages at $a = 0.05$ affected by: (a) acid scarification [$F(3,48) = 858.072$, $P = 0.000$], (b) $GA_3$ [$F(3,48) = 199.627$, $P = 0.000$] and (c) the interaction between the factors acid scarification and $GA_3$ [$F(9.48) = 23.727$, $P = 0.000$]. The main effects of two factors are not analyzed since there is significant interaction between them. 30, 60 and 90 min scarified seeds, prior to $GA_3$ application (500, 1000, 2000 ppm), resulted in higher germination percentages compared to non-scarified seeds that were treated with $GA_3$ ($p < 0.05$) (Table 2). In treatments with 500 and 1000 ppm $GA_3$, seeds scarified for 60 and 90 min gave higher germination percentages than seeds scarified for 30 min

**Table 1. Mean germination percentages (GP) of *P. spina-christi* seeds of the four periods of cold stratification (CS) in each of the four durations of acid scarification (AS) (interaction between the factors 'AS' and 'CS' – simple main effects).**

| CS, weeks | 0 min AS | 30 min AS | 60 min AS | 90 min AS |
|---|---|---|---|---|
| | GP, % ± S.D. | GP, % ± S.D. | GP, % ± S.D. | GP, % ± S.D. |
| 0 | 4.17 a **b** ± 1.67 | 10.00 b **b** ± 2.72 | 57.50 b **a** ± 4.19 | 60.00 b **a** ± 4.71 |
| 4 | 3.33 a **b** ± 2.72 | 84.17 a **a** ± 3.19 | 85.00 a **a** ± 4.30 | 83.33 a **a** ± 4.71 |
| 8 | 3.33 a **b** ± 2.72 | 85.00 a **a** ± 4.30 | 82.50 a **a** ± 4.19 | 78.33 a **a** ± 6.39 |
| 12 | 6.67 a **b** ± 2.72 | 80.00 a **a** ± 5.45 | 79.17 a **a** ± 5.69 | 75.84 a **a** ± 6.31 |

Means, in a column are statistically different at $p<0.05$, when they share no common letter (letters in normal print). The comparisons were made using the Bonferroni test.

Means, in a row are statistically different at $p<0.05$, when they share no common letter (letters in bold print). The comparisons were made using the Bonferroni test.

($p<0.05$). In treatment with 2000 ppm $GA_3$, seeds scarified for 90 min gave higher germination percentages than seeds scarified for 30 min ($p<0.05$). For seeds that were not scarified, treatment with 2000 ppm $GA_3$ gave higher

**Table 2. Mean germination percentages (GP) of *P. spina-christi* seeds of the four concentrations of gibberellic acid ($GA_3$) in each of the four durations of acid scarification (AC) (interaction between the factors 'AC' and '$GA_3$'–simple main effects).**

| $GA_3$, ppm | 0 min AS | 30 min AS | 60 min AS | 90 min AS |
|---|---|---|---|---|
| | GP, % ± S.D. | GP, % ± S.D. | GP, % ± S.D. | GP, % ± S.D. |
| 0 | 4.17 b **c** ± 1.67 | 10.00 c **b** ± 2.72 | 57.50 b **a** ± 4.19 | 60.00 b **a** ± 4.71 |
| 500 | 6.67 ab **c** ± 2.72 | 63.33 b **b** ± 4.71 | 90.00 a **a** ± 4.71 | 88.33 a **a** ± 4.30 |
| 1000 | 9.17 ab **c** ± 3.19 | 80.00 a **b** ± 3.85 | 90.83 a **a** ± 3.19 | 90.00 a **a** ± 2.72 |
| 2000 | 10.83 a **c** ± 3.19 | 81.67 a **b** ± 4.30 | 88.33 a **ab** ± 4.30 | 90.84 a **a** ± 4.19 |

Means, in a column, are statistically different at $p<0.05$, when they share no common letter (letters in normal print). The comparisons were made using the Bonferroni test.

Means, in a row, are statistically different at $p<0.05$, when they share no common letter (letters in bold print). The comparisons were made using the Bonferroni test.

germination percentage than treatment without $GA_3$ ($p<0.05$). In each of the 3 durations (30, 60 and 90 min) of immersion in sulphuric acid the seeds that were treated with $GA_3$ gave higher germination percentages than the seeds that weren't treated with $GA_3$ ($p<0.05$). In 30 min scarified seeds, treatment with 1000 and 2000 ppm $GA_3$ gave higher germination percentages than treatment with 500 ppm $GA_3$ ($p<0.05$). In 60 or 90 min scarified seeds, no significant differences were observed in germination percentages among treatments with 500, 1000 or 2000 ppm $GA_3$ ($p>0.05$) (Table 2).

In total of the 28 treatment combinations that were conducted, those that gave the best 10 germination percentages are presented in Table 3. The combination of acid scarification for 60 or 90 min and gibberellic acid (regardless of concentration) treatments gave high germination percentages that didn't differ significantly with the combinations of 60 min acid scarification plus 4 weeks cold stratification and of 30 min acid scarification plus 8 weeks cold stratification (Table 3).

## Discussion

In this study, the increase of scarification duration from 30 to 60 or 90 min, without any other treatment, caused an analogous significant increase in germination percentages of *P. spina-christi* seeds. Germination percentage of non-scarified seeds was very low. This indicates that *P. spina-christi* seed have a hard, impermeable seed coat (physical dormancy) and that a 30 min acid scarification is not enough to erode the seed coat. This is in agreement with recommendations of Takos et al. (2001) that *P. spina-christi* seeds require scarification in order to germinate. They found that the increase of duration of acid scarification (30, 60, 120 and 240 min) caused an increase in germination percentages (36, 63, 69, and 92% respectively). Also, in their review regarding seed propagation of mediterranean trees and shrubs Piotto et al. (2003) recommended sulphuric acid scarification for 40 to 120 minutes to erode the seed coat. Olmez et al. (2007) reported that treatment of seeds with sulphuric acid for 40 minutes gave the best ger-

**Table 3. Treatments that gave the best ten mean germination percentages (GP) of *P. spina-christi* seeds. These treatments were combinations between a) acid scarification (AS) and cold stratification (CS) and b) acid scarification (AS) and gibberellic acid ($GA_3$).**

| Treatments | GP, % ± S.D. | Treatments | GP, % ± S.D. |
|---|---|---|---|
| 90 min AC + 2000 ppm $GA_3$ | 90.84 a ± 4.19 | 60 min AC + 2000 ppm $GA_3$ | 88.33 abc ± 4.30 |
| 60 min AC + 1000 ppm $GA_3$ | 90.83 ab ± 3.19 | 60 min AC + 4 weeks CS | 85.00 abc ± 4.30 |
| 60 min AC + 500 ppm $GA_3$ | 90.00 abc ± 4.71 | 30 min AC + 8 weeks CS | 85.00 abc ± 4.30 |
| 90 min AC + 1000 ppm $GA_3$ | 90.00 abc ± 2.72 | 30 min AC + 4 weeks CS | 84.17 bc ± 3.19 |
| 90 min AC + 500 ppm $GA_3$ | 88.33 abc ± 4.30 | 90 min AC + 4 weeks CS | 83.33 c ± 4.71 |

Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using the Duncan test.

mination percentages under both greenhouse and open field conditions (65.05 and 54.99% respectively). In contrast, they found that the increase of the duration of acid scarification (from 80 to 120 min) caused a significant decrease in germination percentages under both greenhouse and open field conditions.

In this study, germination percentages of seeds that were subjected only to cold stratification for 4, 8, and 12 weeks were very low. Similar results were reported by Olmez et. al. (2007) where cold stratification of seeds for 20, 40 and 60 days gave the lowest germination percentages under both greenhouse and open field conditions (11.42–13.47% and 3.37–8.99% respectively). In contrast, in their review Piotto et al. (2003) recommended cold moist stratification for 4 to 5 months. Takos et. al. (2001) reported that cold stratification treatment (for 2, 3 and 4 months) had the same positive effect in seed germination as acid scarification. They found that seeds stratified for 2, 3 or 4 months gave germination percentages equal to 63, 56 and 79% respectively. Possibly, during the breaking of the hard exocarp into the hammer mill that Takos et al. (2001) used for the seed extraction, the seed coat of some seeds had cracked. Consequently, these seeds germinated after cold scarification treatment.

According to the results of this study the combination of acid scarification and cold stratification treatments was necessary to maximize germination of *P. spina-christi* seeds. Cold stratification for 4 weeks of scarified seeds was enough to maximize germination. Possibly, the seeds of *P. spina-christi*, apart from the hard seed coat also have a nondeep physiological dormancy. According to Baskin and Baskin (1998) nondeep physiological dormancy in some species is broken by relative short periods of cold stratification. Takos et al. (2001) reported that seeds that were acid scarified for 30 min and then followed by 2, 3 or 4 months cold stratification gave high germination percentages.

$GA_3$ treatments significantly increased the germination of scarified seeds. Germination percentages of seeds that were scarified for 60 or 90 min and were then treated with $GA_3$ were very high. This implies that $GA_3$ treatment substituted cold stratification requirement in *P. spina-christi* seeds. Also, Baskin and Baskin (1998) reported that GA stimulates germination of seeds with nondeep physiological dormancy.

## Conclusions

The results of this study revealed that acid scarification treatment was stronger than cold stratification in breaking the dormancy of *P. spina-christi* seeds. Cold stratification of acid scarified seeds for 4 weeks maximizes the germination of *P. spina christi* seeds. However, similar results as well as overcoming seed dormancy in 24 hours could be achieved by treatment of scarified seeds with $GA_3$.

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