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Seed treatments to break dormancy and stimulate germination in *Cercis siliquastrum* L. and *Carpinus orientalis* Mill.

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The aim of this study was to investigate the methods that increase the germination of the native species *Cercis siliquastrum* and *Carpinus orientalis*, whose seeds exhibit dormancy. Seeds of *C. siliquastrum* were collected from a natural habitat and an urban environment, and seeds from both origins were given 6 different dormancy-breaking treatments. Seeds of *C. orientalis* were collected from an urban environment and were given 5 different dormancy-breaking treatments. For *C. siliquastrum* seeds, the treatment that gave the best final germination was the combination of acid scarification for 30 min and immersion in 500 ppm gibberellin (GA_3) (52% for the seeds from the urban environment and 62.5% for the seeds from the natural habitat germinated). For *C. orientalis* seeds, the results showed that, the most effective treatment was moist-cold stratification for 12 weeks which resulted in 84% germination.

Key words: *Carpinus orientalis, Cercis siliquastrum,* dormancy, gibberellin, scarification, stratification.

INTRODUCTION

Cercis siliquastrum L. and *Carpinus orientalis* Mill. are both native to South eastern Europe and South-west Asia (Boratynski et al., 1992; Pijut, 2008) and are prevalent throughout continental Greece.

C. siliquastrum L. has a potential use for landscaping due to its ornamental features (blackish bark, heart-shaped leaves and bright purplish-rose flowers) and for borders, erosion control, windbreaks and wildlife plantings (Gebre and Karam, 2004; Unal et al., 2009) and as a medical plant (Attard, 2002). Furthermore, the species is well adapted to semi-arid conditions and is tolerant of air pollution and nutrient deficient soils (Gebre and Karam, 2004; Zencirkiran et al., 2010). *C. orientalis* Mill. is also an attractive tree with many ornamental attributes (excellent form, twisted branches and glossy leaves, make fantastic hedges, forest green foliage throughout the season). It is highly tolerant of urban Pollution and will even thrive in inner city environments. Its wood is extremely hard Change and is used for making tool handles and mallet heads and to produce the high-quality charcoal used in gunpowder manufacture (Pijut, 2008). Moreover, it is traditionally used as a fodder in the Mediterranean region (Papanastasis et al., 1997).

Conventional propagation of *C. siliquastrum* L. and *C. orientalis* Mill. is most commonly from seed. However, the seeds of both species exhibit dormancy (Piotto et al., 2001), and their germination is not always ensured. *C. siliquastrum* seed exhibits a double dormancy, a physical (hard and impermeable seed coat) and physiological (embryo) dormancy and generally requires special treatment to overcome dormancy (Rascio et al., 1998; Unal et al., 2009; Zencirkiran et al., 2010). Three treatments have proven satisfactory for overcoming *Cercis* L. seed coat impermeability: mechanical

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scarification; immersion in sulphuric acid; or in hot water. Acid treatment has generally produced more consistent or slightly better results (Liu et al., 1981). *C. orientalis* seed exhibits mainly physiological dormancy; the dormancy caused by conditions in the embryo and endosperm and may be overcome by cold stratification treatments (Pijut, 2008; Piotto et al., 2001).

The purpose of this research was to: break the seed dormancy of *C. siliquastrum* and *C. orientalis* using different treatments, describe the effects of treatments on the germination of *C. siliquastrum* and *C. Orientalis*, and finally to propose treatments that maximize seed germination of the above species.

MATERIALS AND METHODS

Seed collection and preparation

Mature pods of *C. siliquastrum* were collected in August 2009 from a natural habitat (Kassandra Chalkidiki, N. Greece) and from an urban environment (centre of Thessaloniki, N. Greece). The legumes were dried in shade for 24 h (Zencirkiran et al., 2010) and then, were grated by hand to break them open to release the seeds. Sieving and flotation were used to clean the seeds. Mature fruits of *C. orientalis* were collected in early December 2009 from the botanic garden of the Faculty of Forestry and Natural Environment of Aristotle University in Thessaloniki. After collection, fruits were put to dry in thin layers in fresh, well-aerated facilities. De-winging was done by hand. Afterwards all the cleaned seeds of both species were stored in glass containers in the refrigerator (4^oC). Prior to experiments, all seeds were soaked in water at room temperature for 24 h and any floated seeds were recorded and excluded from the experiments.

Viability test

To determine the viability of *C. siliquastrum* and *C. orientalis* seeds, the topographical tetrazolium test of viability was used. For each species, 100 seeds were used in 4 replicates of 25 seeds (ISTA, 1993). The seeds were soaked for 20 h in tap water. Pre-moistening is necessary because staining is more intense and imbibed seeds are generally less fragile than dry seeds. Following cutting (transversely or longitudinally), seeds were immersed in a 1% triphenyl tetrazolium chloride (TTC), (Takos and Efthimiou, 2003; Bonner and Karrfalt, 2008), where they remained for 24 h in dark, at room temperature. The embryos with red coloured radicle and cotyledons were considered to be viable (ISTA, 1999; Bonner, and Karrfalt, 2008).

Applied treatments

Treatments applied for *C. siliquastrum* seeds were:

a) Mechanical scarification by sand paper,
b) Soaking in concentrated (98%) sulfuric acid (H_2SO_4) for 20 min
c) For 30 min,
d) Soaking in concentrated (98%) H_2SO_4 for 30 min, and immersion in 500 ppm gibberellin (GA_3) for 24 h in the dark at 25°C,
e) Immersion in 500 ppm GA_3 for 24 h in the dark at 25°C,
f) Immersion in hot water (70 to 80°C) for 1 h,

Treatments applied for *C. orientalis* seeds were:

a) Mechanical scarification by sand paper,
b) Soaking in concentrated (98%) H_2SO_4 for 30 min and moist-cold stratification (at 4°C) for 12 weeks,
c) Soaking in concentrated (98%) H_2SO_4 for 1 h and moist-cold stratification (at 4°C) for 12 weeks,
d) Moist-cold stratification (at 4°C) for 12 weeks,
e) Immersion in 500 ppm GA_3 for 24 h in the dark at 25°C. After each treatment, the seeds were rinsed with distilled water.

Germination tests

After the application of treatments, seeds of the 2 species were placed in plastic Petri dishes (9 cm in diameter) on sterilized sand, moistened with distilled water and fungicide to avoid fungi development. For each species and treatment, there were 8 replications of 25 seeds (Teketay, 1996; Bonner and Karrfalt, 2008).

Petri dishes were randomly arranged on the shelves of the growth chamber (alternating temperature 25/20°C in photoperiod 8 h light/16 h darkness, respectively and humidity 80%) (Piotto et al., 2001). Sand in the Petri dishes was watered as needed with distilled water to ensure adequate moisture for seed germination. Criterion of germination was the appearance of the radicle, at least 2 mm long, according to the ISTA rules (ISTA, 1999). Germinated seeds were recorded every 3 days for 2.5 months, and at the end of the tests, the final germination percentages (%) for each treatment and species were calculated.

Statistical analysis

Statistical analysis was carried out using SPSS 19.0 (SPSS, Inc., USA). Data were checked for assumptions of normality and homogeneity of variances. Germination percentages were transformed to arcsine square root values (Snedecor and Cochran, 1988) and reanalyzed. One-way analysis of variance (ANOVA) was applied, and the comparisons of means were made using Duncan's test at significance level $P \leq 0.05$ (Norusis, 1994).

RESULTS AND DISCUSSION

C. siliquastrum

The percentage of the healthy and complete seeds originated either from a natural habitat (Kassandra Chalkidiki) or from an urban environment (Thessaloniki city) was 99% (data are not shown in the tables). The viability test of *C. siliquastrum* seeds originating either from Thessaloniki or Chalkidiki showed that, the percentage of embryos that stained entirely red was 75%, while the percentage of embryos with stained cotyledons was 85 to 90% (data not shown). Similar results were found by Zencirkiran et al. (2010) who studied *C. siliquastrum* seeds which originated from a natural habitat in Bursa-Turkey.

The seeds originating from Chalkidiki generally had higher germination ability and their germination started earlier than that of seeds originating from Thessaloniki (Table 1, Figure 1A and B). The treatments applied to break dormancy significantly affected seed germination. Seeds of both origins subjected to mechanical

Table 1. Final germination percentages in 6 treatments applied in seeds of *C. siliquastrum* from an urban environment (Thessaloniki city) and a natural habitat (Kassandra Chalkidiki).

Treatment	Germination (%) (Thessaloniki city)	Germination (%) (Kassandra Chalkidiki)
Mechanical scarification by sand paper	5.5 (2.38)^{cb}	8 (2.62)^{cb}
Soaking in concentrated (98%) (H_2SO_4) for 20 min	2 (1.07)^{c}	4 (2.51)^{cb}
Soaking in concentrated (98%) (H_2SO_4) for 30 min	4 (1.31)^{c}	1 (0.66)^{c}
Soaking in concentrated (98%) (H_2SO_4) for 30 min and immersion in 500 ppm GA_3 for 24 h in the dark at 25°C.	52 (4.78)^{a}	62.5 (6.10)^{a}
Immersion in 500 ppm (GA_3) for 24 h in the dark at 25°C.	13 (3.68)^{b}	14.5 (1.99)^{b}
Immersion in hot water (70 to 80°C) for 1 h	0 (0.00)^{c}	0 (0.00)^{c}

Values are means ± SE of the mean (in brackets). Within the same column means followed by different letter are significantly different ($P < 0.05$).

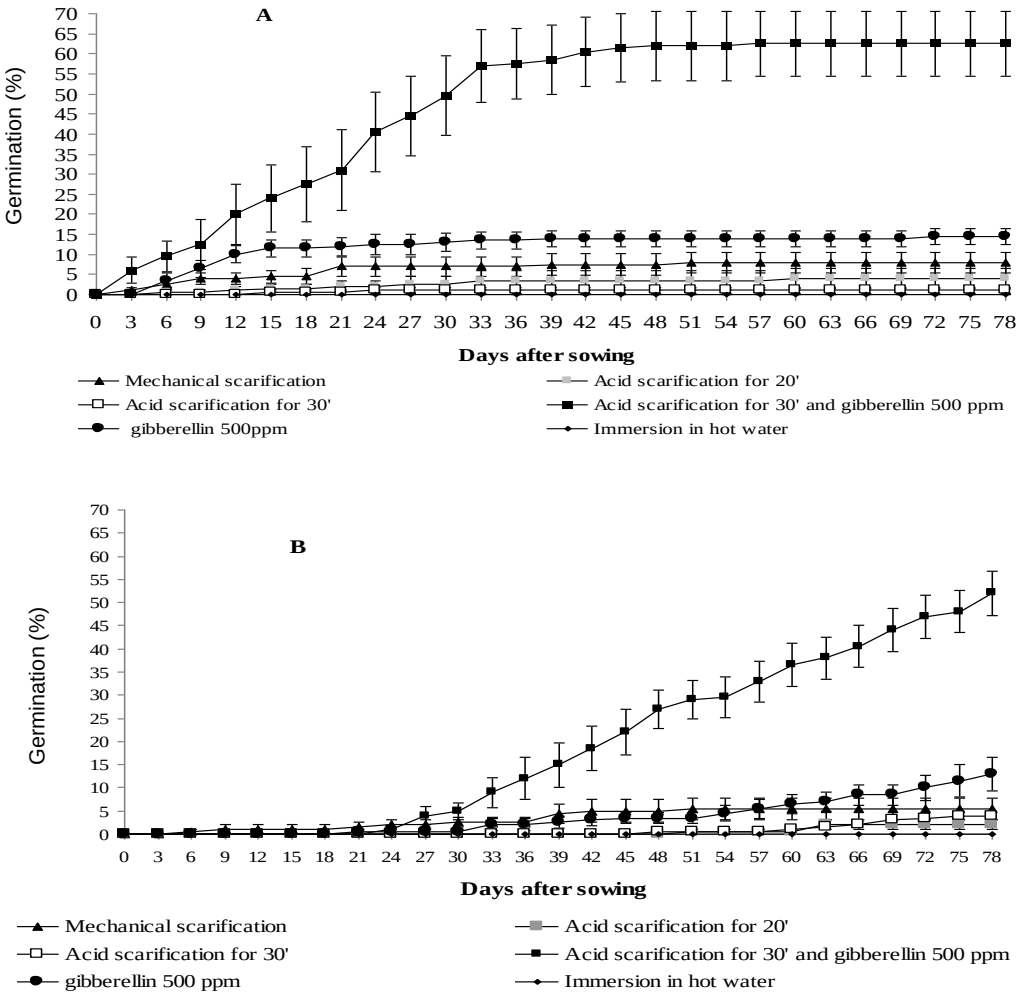

Figure 1. Germination rate (%) of *Cercis siliquastrum* seeds originated from a natural habitat, A; from an urban environment, B; subjected to various breaking dormancy treatments.

Table 2. Final germination percentages in 5 treatments applied in seeds of *C. orientalis*

Treatment	Germination (%)
Mechanical scarification by sand paper	2.5 (1.05)[b]
Soaking in concentrated (98%) (H_2SO_4) for 30 min and moist-cold stratification (at 4°C) for 12 weeks.	0 (0.00)[b]
Soaking in concentrated (98%) (H_2SO_4) for 1 h and moist-cold stratification (at 4°C) for 12 weeks.	0 (0.00)[b]
Moist-cold stratification (at 4°C) for 12 weeks.	84 (3.70)[a]
Immersion in 500 ppm (GA_3) for 24 h in the dark at 25°C.	4 (1.51)[b]

Values are means ± SE of the mean (in brackets). Means followed by different letter are significantly different ($P < 0.05$).

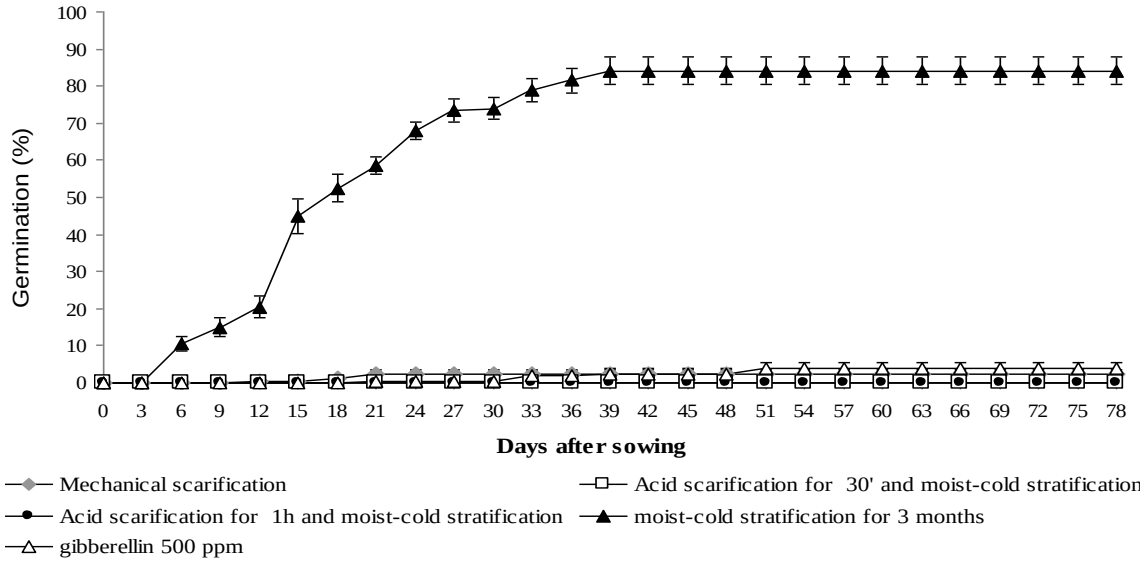

Figure 2. Germination rate (%) of *Carpinus orientalis* seeds subjected to various breaking dormancy treatments.

scarification had very low germination (5.5 and 8%), while none of the seeds immersed to hot water for 1 h germinated (Table 1). Gebre and Karam (2004) found also that, the seeds subjected to mechanical scarification did not germinate. Acid scarification treatments (for 20 and 30 min) did not break dormancy since the germination percentages of seeds from both origins ranged from 1 to 4%, (Table 1), proving the existence of physiological (embryo) dormancy. Similarly, Zencirkiran et al. (2010) reported a germination capacity of between 0.35 and 0.60% for *C. siliquastrum* seeds subjected to chemical scarification with H_2SO_4 for 15 and 30 min, and they concluded that, the combination of the chemical scarification with the cold stratification was required to break seed dormancy. Martinucci et al. (1985) attributed the embryo dormancy in the existence of ferulic acid in endosperm, which is probably responsible for the reduction of availability of oxygen in the embryo.

On the other hand, it seemed that the 500 ppm GA_3 contribute more to stimulating germination than previous treatments. However, the optimal treatment that gave the highest germination percentage among all the treatments was the combination of acid scarification for 30 min with 500 ppm GA_3. Under this treatment, seeds originated from Thessaloniki presented 52% final germination capacity but the germination was delayed (started after 24 days), while seeds from Chalkidiki presented higher germination capacity of 62.5% and the germination started much earlier; after 6 days (Figure 1A and B).

C. orientalis

The percentage of the healthy and complete seeds was only 20% (data are not shown in the tables). The viability test of *C. orientalis* healthy seeds showed that, the

percentage of embryos which were stained entirely red was 80% while the percentage of embryos with stained cotyledons was 90 to 95% (data are not shown in the tables). Treatments applied significantly affected the germination behavior. Seeds treated with mechanical scarification and those immersed in 500 ppm GA_3 presented very low final germination capacity (Table 2). Other researchers proposed the combination of GA_3 with cold stratification or scarification to break the dormancy of *Carpinus caroliana* (Bonner and Karrfalt, 2008).

Seeds subjected to acid scarification, for 30 min and 1 h, in combination with moist-cold stratification for 12 weeks did not germinate at all. This proves that, *C. orientalis* seeds do not exhibit physical dormancy, and moreover the acid duration may cause embryo damage. On the other hand, seeds subjected to moist-cold stratification had significantly higher germination capacity (84%), and the germination started much earlier (within 6 days) than in the other treatments (Table 2 and Figure 2)

Similar result was also reported by Takos et al. (2001). Also, Piotto et al. (2001) reported that, if fresh seeds (collected during the autumn) are going to be used, 3 months cold of stratification are enough to remove dormancy and the final germination is fluctuated from 80 to 85%. Hartmann et al. (1997) proposed for *Carpinus* seeds either autumn sowing and no treatment or spring sowing after cold stratification for 3 to 4 weeks.

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