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Effect of acid scarification and cold moist stratification on the germination of *Cercis siliquastrum* L. seeds

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Abstract: Dormancy in *Cercis siliquastrum* seeds is due to the hard, impermeable seed coat and inhibition by endosperm. The effects of acid scarification, cold moist stratification, and the combination of both on breaking dormancy and enhancing seed germination were evaluated. Seeds were scarified with concentrated (95%-97%) sulfuric acid for various times (0, 20, 40, and 60 min), followed by cold moist stratification for 0, 1, 2, 3, or 4 months. Unscarified seeds did not germinate whether they were stratified (up until 4 months) or not. Similarly, seeds that were scarified (20, 40, and 60 min) and then stratified for 0 or 1 month did not germinate or exhibited very low germination percentages. The interaction between acid scarification and cold stratification treatments significantly affected seed germination. Particularly, after a period of 2 months of cold stratification, increasing the duration of scarification (20 to 60 min) also increased the germination percentages (31% to 65%). High germination percentages equal to 94%, 88%, and 98% were attained after a period of 3 months of cold stratification for seeds that had been scarified for 20, 40, and 60 min, respectively. Longer periods of stratification (4 months) of seeds scarified for 20, 40, and 60 min reduced the germination percentages (81%, 68%, and 59%, respectively). This decrease was higher in seeds that were scarified for 60 min.

Key words: Acid scarification, *Cercis siliquastrum*, cold stratification, dormancy, germination

Introduction

Cercis siliquastrum L. (Judas tree) is a leguminous deciduous shrub or small tree (up to 10 m high) native to southeastern Europe and south and west Asia (Boratynski et al. 1992; Brickell 1996). It is a prevalent species throughout continental Greece, on the northern Aegean and on the northern Ionian Islands. *C. siliquastrum* can be found mostly in valleys of streams and rivers in maquis communities, on the borders of broadleaved or coniferous forests (Boratynski et al. 1992).

C. siliquastrum is produced by the nursery industry mainly for ornamental use, due to attractive flowers that appear before the foliage emerges in spring. Moreover, this deciduous species may also be used in reforestation of disturbed lands to improve the landscape. *C. siliquastrum*, like many other woody plants, is propagated from seeds, as this method is cost-effective. This propagation technique exhibits difficulty due to seed dormancy. The seed of this species, as in many Leguminosae, has a hard seed coat that is impermeable to water (physical dormancy).

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Riggio-Bevilacqua et al. (1985) attributed the inhibition of water uptake to the combination effect of the imperviousness of the hilum and the impermeability of a noncellular lipid layer found between the inner surface of the integument and the endosperm. However, in contrast to other woody legumes that display only seed coat dormancy, in *C. siliquastrum* seed dormancy also depends on chemical inhibitors that are found in the endosperm (Martinucci et al. 1985). The propagator must carry out pregermination treatments to overcome seed dormancy, so that the highest percentage of viable seed is brought to the point of germination. Recommendations for seed germination of *C. siliquastrum* include scarification followed by cold stratification (Rascio et al. 1998; Piotto et al. 2003; Gebre and Karam 2004). Seeds immersed in concentrated sulfuric acid (acid scarification) have been used to break seed coat dormancy in species of *Cercis*. According to Gebre and Karam (2004), scarification with concentrated (1.84 g cm^{-3} specific gravity) sulfuric acid for 15 min proved to be quite satisfactory in breaking the hard seed coat in *C. siliquastrum*. Jones and Geneve (1995) and Geneve (1991) considered scarification with concentrated sulfuric acid for 30 min for seeds of *C. canadensis* ample in order to allow for water imbibition. Liu et al. (1981) also recommended scarification with concentrated sulfuric acid for 30-90 min to break the hard coat of *C. canadensis* seeds. After scarification, the seed of *C. siliquastrum* requires a period of cold moist stratification in order to remove endosperm dormancy (Rascio et al. 1998). Gebre and Karam (2004) considered the most effective and necessary treatment for germination of imbibed *C. siliquastrum* seeds to be moist stratification at 4 °C for 16 weeks. Liu et al. (1981) recommended that cold stratification is necessary for germination of scarified *C. canadensis* seeds. The cold stratification period (at 5 °C) that they used to overcome dormancy was 60 days. In contrast, Hamilton and Carpenter (1975) reported that seed dormancy in *C. canadensis* was only controlled by the seed coat and that cold stratification was not necessary.

In the published literature, there is no description of the effect of various durations of acid scarification followed by cold stratification for different periods on seed germination. The knowledge of the exact treatment that maximizes the seed germination of *C. siliquastrum* will result in a successful propagation by the nursery industry. The purpose of this research was to 1) break the seed dormancy of *C. siliquastrum* using treatments combining acid scarification and cold moist stratification, 2) describe the effects of acid scarification and cold stratification treatment combinations on the germination of a single *C. siliquastrum* seed lot, and 3) propose treatments that maximize germination of *C. siliquastrum* seeds.

Materials and methods

Mature fruits (legumes) of *C. siliquastrum* were collected in August 2003 from a number of trees (more than 10) growing in their natural habitat (39°20′36″N, 20°34′42″E, 50 m elevation) in western Greece. The legumes were first dried under the sun and were then grated by hand in order to break them. Sieving and flotation were used to clean the seeds. Flotation removed trash (parts of legumes) and empty, broken, and insect-damaged seeds. The clean seeds were then spread upon filter paper and left to dry. After drying, the seeds were stored in glass containers in the refrigerator (2-4 °C). The experiment was conducted the following winter.

Seed treatment

The germination experiment was conducted in the Laboratory of Silviculture, Faculty of Forestry and Natural Environment, Aristotle University of Thessaloniki. Seeds were put in a glass container with concentrated (95%-97%) sulfuric acid and gently stirred periodically. The durations of immersion in acid were 0, 20, 40, and 60 min. After the treatment, the seeds were removed from the acid and were washed thoroughly under running water. Scarified and unscarified seeds were mixed with wet sterilized river sand and placed in plastic containers, and they then underwent cold stratification at 2-4 °C for 0, 1, 2, 3, or 4 months. Each container corresponded to a different duration of immersion in sulfuric acid (there were 4 plastic containers). In total, 20 treatments (combinations of acid scarification (AS) and cold stratification (CS)) were applied. During CS, moisture was being checked, and water was added when necessary.

Germination test

At the end of each CS period, a random sample of 100 seeds was taken from each plastic container and randomly placed in 4 petri dishes (25 seeds per petri dish). For each treatment (20 in number), there were 4 replications of 25 seeds. The seeds were placed on sterilized river sand moistened with distilled water in 9 cm plastic petri dishes. Prior to the arrangement of seeds in the 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 (Sherer Controlled Environment Lab., Model MG 4 – 4) was set to 20 °C for a 16 h dark period and 25 °C 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. Each germinated seed was removed from the dishes in order to avoid confusion in counting. Petri dishes were watered as needed with distilled water to ensure adequate moisture for seed germination. Finally, the germination percentage was calculated for each replication. The germination percentage of each treatment was calculated from the average of the 4 replications' percentages.

Statistical analysis

The experimental design was a completely randomized design with 2 factors. The factors were the duration of AS and the length of the CS period. The germination data of unscarified (0 min) seeds was not analyzed since, regardless of the duration of CS, none of the seeds germinated in this pretreatment. Similarly, the seeds that were not stratified, regardless of the duration of AS, did not germinate. Moreover, seeds scarified for 20, 40, or 60 min followed by CS for 1 month showed very low germination percentages (0%, 1%, and 10%, respectively). Therefore, in the statistical analysis, the levels of the factors were 20, 40, and 60 min for AS, and 2, 3, and 4 months for CS (a 3×3 factorial design). The data were analyzed using ANOVA in the frame of the GLM procedure (general linear model) (Gomez and Gomez 1984). Data were checked for assumptions of normality and homogeneity of variances (Sokal and Rohlf 1995). When the assumptions were violated, the germination percentage data were transformed to arcsine square root values (Snedecor and Cochran 1988) and reanalyzed. Treatment means were separated using Duncan's test (Klockars and Sax 1986; Matis 1989) at significance level $P \leq 0.05$. All statistical analysis was carried out using SPSS 12.0 (SPSS, Inc., USA).

Results

Effects of AS on mean seed germination rate varied significantly across different levels of CS. Additionally, the main effects of AS and CS were significant.

All AS treatments (20, 40, and 60 min) exhibited the highest seed germination after a period of 3 months CS (Table 1). Furthermore, in each AS treatment, when the duration of CS was increased from 2 to 3 months, a significant increase in seed germination was observed. After a period of 4 months of CS, a significant decrease in seed germination for all AS treatments was observed.

The effect of AS varied among CS periods (Table 1). After a period of 2 months of CS, seeds that had been subjected to AS for 60 min exhibited the highest germination percentage compared to those of seeds that had been scarified for 20 or 40 min. On the contrary, after a period of 4 months of CS, seeds that had been subjected to AS for 20 min exhibited a germination percentage that was higher than the germination percentages of seeds that had been subjected to AS for 40 or 60 min. In the case of a 3-month CS period, there was no significant difference in germination percentages between seeds that had been subjected to minimum and maximum times of AS.

The optimal treatments that gave the highest germination percentage among all the treatments were 60 or 20 min of AS followed by 3 months of CS.

Discussion

As mentioned in the statistical analysis section, unscarified seeds subjected to CS for 1, 2, 3, or 4 months did not germinate, indicating that *C. siliquastrum* seeds have the typical hard, impermeable

Table 1. Mean germination percentages of *C. siliquastrum* seeds of all acid scarification and cold stratification treatment combinations.

Acid scarification (min)	Cold stratification (months)	Germination percentage (%, ± S.D.)
20	2	31 d[1] ± 5.03
	3	94 a ± 5.16
	4	81 b ± 6
40	2	38 d ± 4
	3	88 b ± 5.66
	4	68 c ± 5.66
60	2	65 c ± 6
	3	98 a ± 2.31
	4	59 c ± 5.03

[1]Means sharing the same letters are not statistically different (P > 0.05).

seed coat (physical dormancy) of the leguminous species. This is in agreement with the results of research by Gebre and Karam (2004), which reported that unscarified seeds of *C. siliquastrum* did not exhibit any imbibition or germination as a result of the hard seed coat. The hardness and impermeability of the seed coat, as an inhibited factor in seed germination, has been studied in several species of the family Leguminosae (Demel 1996; Sacheti and Al-Rawahy 1998; Sy et al. 2001; Orozco-Almanza et al. 2003; Pipinis et al. 2005). Similarly, seeds scarified with concentrated sulfuric acid for 20, 40, or 60 min that were not cold stratified did not germinate, indicating that *C. siliquastrum* seeds also exhibited endogenous dormancy. Martinucci et al. (1985) attributed the endogenous dormancy in the appearance of ferulic acid to the endosperm, which is probably responsible for reducing oxygen availability to the embryo. CS treatment for 1 month for scarified seeds also resulted in very low seed germination (up to 10%). This indicated that a 1-month CS period for scarified seeds was not sufficient to reduce the effect of the inhibitory substances of the endosperm. Gebre and Karam (2004) reported that mechanically scarified seeds of *C. siliquastrum* did not germinate without CS. They also observed a very low germination percentage (7%) after an 8-week CS period of mechanically scarified seeds. One reason for the existence of double dormancy in *C. siliquastrum* seeds is that the seeds, after maturation and dispersal subject to natural conditions (fluctuating temperature, effects of microorganisms or animals, fire), can overcome seed coat dormancy. If the seed coat becomes permeable, seeds can be cold stratified during the winter and germinate the following spring.

According to the results of our study, the combination of AS and CS treatments (for more than 1 month) was necessary to break dormancy and to enhance germination of *C. siliquastrum* seeds. Tipton (1992) reported that seeds of *C. canadensis* var. *mexicana* did not germinate without both scarification and CS. Cold stratification of acid scarified seeds (20-60 min) for 3 months appeared to be sufficient to maximize germination percentage (88%-98%). Gebre and Karam (2004) recommended the most effective CS period of mechanically scarified seeds to be 16 weeks. In our research, *C. siliquastrum* seeds required a shorter CS period than that recommended by Gebre and Karam (2004). This was possibly the result of the different scarification method that Gebre and Karam used, or the variation in the degree of seed dormancy among plants of the same species that grow in different environmental conditions (Fenner 1991; Anderson and Milberg 1998; Cavieres and Arroyo 2000; Rosner et al. 2003; Fenner and Thompson 2005). It is interesting to note that in acid scarified seeds treated with 2 months of CS, an increase in germination percentages (from 31% to 65%) was observed as the duration of immersion in sulfuric acid increased from 20 to 60 min. The

immersion of seeds in sulfuric acid for 60 min, except for the breaking of the seed coat, perhaps allowed the endosperm changes that are needed for dormancy release to begin earlier during CS. Rascio et al. (1998) reported that 60 days of CS in seeds that had been subjected to scarification with a razor blade led to about 65% of germination. For *C. canadensis*, the seed germination was 67%-72% after 30, 60, or 90 min of sulfuric acid scarification followed by a 60-day stratification period at 5 °C (Liu et al. 1981). The increase of the CS period up to 3 months led to a positive seed germination response, irrespective of the immersion time in sulfuric acid. A 3-month CS period of scarified seeds, regardless of the AS duration, was enough to overcome the endosperm dormancy. The increased concentration of gibberellic acid in seeds, as a result of a 3-month CS period (Powell 1987), perhaps counteracted the inhibitory effect of ferulic acid in endosperm (Martinucci et al. 1985). A longer CS period decreased the germination percentages of scarified seeds. The negative influence of a 4-month CS period in seed germination was larger when the duration of immersion in sulfuric acid increased. After a long duration of AS treatment, CS for 4 months may cause seed damage. Gebre and Karam (2004) observed a significant reduction in the germination of mechanically scarified *C. siliquastrum* seeds after a period of 20 weeks of CS.

In conclusion, the germination of *C. siliquastrum* seeds can be achieved only by scarification followed by cold moist stratification treatment. The length of the CS period (over 1 month) determines the duration of immersion in sulfuric acid that is required for the maximum seed germination. In this article, a single seed lot was used to demonstrate the efficacy of treatment combinations (acid scarification and cold moist stratification) on germination of *C. siliquastrum* seeds. In an untested seed lot, considering the variety of degree of seed dormancy among provenances, in order to maximize seed germination the propagator must first determine the duration of each treatment on a small sample before treating the entire lot.

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