This article was listed in Forest Nursery Notes, Summer 2007

127. Effects of some pretreatments on seed germination of nine different drought-tolerant shrubs. Olmez, Z., Gokturk, A., and Temel, F. Seed Science and Technology 35:75-87. 2007.

Olmez, Z., Gokturk, A. and Temel, F. (2007), *Seed Sci. & Technol.*, **35**, 75-87

Effects of some pretreatments on seed germination of nine different drought-tolerant shrubs

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(Accepted April 2006)

Summary

This study was carried out to determine the effects of some pretreatments including soaking in concentrate H_2SO_4, GA_3, floating in hot water and cold stratification and their combinations on seed germination and to investigate how to overcome dormancy of seeds of some drought-tolerant plants. The species used in this study were *Arbutus andrachne* L., *Cistus creticus* L., *Colutea armena* Bois. Huet., *Cotinus coggyria* Scop., *Cotoneaster numullaria* Fisch.&Mey., *Elaeagnus angustifolia* L., *Jasminum fruticans* L., *Paliurus spina-christii* Mill. and *Rhus coriaria* L. The seeds were sown in polyethylene pots under greenhouse conditions and on seedbeds under open field conditions. The statistical approach was a randomized complete block design with three replications. The results (highest germination percentage) for each species were as follow: *C. creticus*; soaking in hot water for 1 minute both under greenhouse (66.89%) and open field (44.89%) conditions, *C. coggyria*; soaking in H_2SO_4 for 20 minutes with cold stratification for 60 days (87.30%) under greenhouse conditions, *C. numullaria*; soaking in H_2SO_4 for 90 minutes with cold stratification for 60 days (53.67%) under greenhouse conditions, *J. fruticans*; cold stratification for 60 days under greenhouse (85,56%) conditions, *E. angustifolia*; soaking in running water at 15°C for 10 days with cold stratification for 30 days both under greenhouse (64.26%) and open field (54.89%) conditions, *Paliurus spina-christii*; soaking in H_2SO_4 for 40 or 80 minutes both under greenhouse (65.05 or 62.85%) and open field (54.99 or 49.38%) conditions, *R. coriaria*; soaking in H_2SO_4 for 30 minutes with cold stratification for 60 days both under greenhouse (54.53%) and open field (39.78%) conditions. None of the treatments that were applied to *A. andrachne* and *C. armena* gave statistically significant improvement over the control.

Introduction

Vegetation cover is one of the most important factors in preventing and controlling soil erosion. It promotes long-term soil surface protection by providing leaf cover that reduces rain-drop effects. In addition, it helps soils to develop a better structure through establishing root system, thereby increasing infiltration and soil stability (Balcı, 1996; Pritchett and Fisher, 1987). Woody vegetation provides better soil protection and lasts longer than annual plants because their roots deepen and improve the soil and the shade they provide facilitates ecosystem metabolism. These functions are essential for ensuring the soil stability and continuity of agricultural activities (FAO, 1989).

Seeds of many woody plant species can not germinate even if they are sown under the correct moisture, oxygen and soil conditions on that year (Ürgenç and Çepel, 2001). This inability to germinate is called seed dormancy and there are different types of seed dormancies that occur due to various reasons. Baskin and Baskin (2004) have classified the

types of seed dormancy as physiological, morphological, morphophysiological, physical and combination dormancies. Some of the biological reasons for dormancy, listed by ISTA (1966 and 1993), are hard and impermeable seed coat, immature or dormant embryo, absence of endosperm and fleshy part of fruit. The degree of seed dormancy varies both among and within species. Poulsen (1996) reported by ascribing to Wolf and Kamondo (1993) that dormancy among and within, seed-lots of the same species varies with provenance, crop year and individual trees. There are different methods and techniques to overcome seed dormancy depending on these factors. For example, in general, such pretreatments like floating on hot water, mechanical or chemical scarification and hot aeration are used for seed coat dormancy while the pretreatments of cold and warm stratifications are applied to dormancy caused by restrictions at the embryo level (Landis *et al.*, 1996).

The most important step in the bio-preventive measures for checking soil erosion is the selection of suitable stabilizing plants. This procedure must also take the climatic and slope conditions into consideration. For example, plant species that develop taproot hold excess water and prevent landslides. In order to achieve effective protection in erosion control areas, the problems should be determined correctly and the required plant species should be chosen accordingly (Ucler *et al.*, 2002).

Occurring in rocky and steep landscapes *Arbutus andrachne* L., *Cistus creticus* L., *Colutea armena* Bois. Huet., *Cotinus coggyria* Scop., *Cotoneaster numullaria* Fisch. & Mey., *Elaeagnus angustifolia* L., *Jasminum fruticans* L., *Paliurus spina-christii* Mill. and *Rhus coriaria* L. are drought-tolerant plants that are important in preventing erosion. These species also effective in increasing the local inhabitants' income level as different parts (e.g. roots, fruits and flowers) of these plants can be used as income generators. According to some researchers, there are germination obstacles in seeds of these species and; thus, there are propagation difficulties (Heit, 1967; Ürgenç, 1986; Piotto *et al.*, 2003). Germination percentages of the seeds of these species varies approximately between 13% and 100% and cold stratification, soaking in H_2SO_4, GA_3 and KNO_3 are well-known methods to increase germination percentage (Belcher and Karrfalt, 1979; Riley, 1981; Kaminski, 1985; Pela *et al.*, 2000; Karam and Al- Salem, 2001).

The aim of the present study was to examine the influence of some pretreatments on seed germination percentages and rates of *A. andrachne*, *C. creticus*, *C. armena*, *C. coggyria*, *C. numullaria*, *E. angustifolia*, *J. fruticans*, *P. spina-christii* and *R. coriaria* seeds.

Material and methods

Ripe fruits of the species were collected from the wild in the Artvin region, located in the Northeastern part of Turkey, between the altitudes of 200 and 1200 m, in August, September and October 2003. The seeds were separated from the fruit material, rinsed in tap water, dried in the shade and stored at +5°C in plastic bags after ratios of full seed were determined.

The pretreatments for each species in the study are listed in table 1. These were applied to determine the effects of these pretreatments on seed dormancy, germination rates (GR) and percentages (GP) for each species.

Table1. Pretreatments for overcoming the seed dormancy.

Species	Pretreatments
Arbutus andrachne	• Cold stratification (CS) for 20, 40 and 60 days • Submersion in 250 mgL-1 GA_3 for 10 and 20 minutes • Control
Cistus creticus	• CS for 20, 40 and 60 days • Soaking in hot water (100°C) for 35 and 60 seconds • Soaking in 50°C water for 180 minutes + 30-day CS • Control
Colutea armena	• CS for 20, 40 and 60 days • Soaking in hot water (100°C) and then allowed to cool for 24 hours • Soaking in tap water for 24 hours • Control
Cotinus coggyria	• CS for 20, 40 and 60 days • Immersion in concentrate (98%) H_2SO_4 for 20, 50 and 80 minutes + 60-day CS • Control
Cotoneaster numullaria	• CS for 20, 40 and 60 days • Immersion in concentrate (98%) H_2SO_4 for 30, 60 and 90 minutes + 60-day CS • Control
Elaeagnus angustifolia	• CS for 20, 40 and 60 days • Soaking in running water (15°C) for 10 days + 30-day CS • Soaking in water for 7 days after snipping off seeds 2 mm both ends • Control
Jasminum fruticans	• CS for 20, 40 and 60 days • Soaking in hot water (100°C) for 30 seconds • Immersion in concentrate (98%) H_2SO_4 for 10 minutes • Control
Paliurus spina-christii	• CS for 20, 40 and 60 days • Immersion in concentrate (98%) H_2SO_4 for 40, 80 and 120 minutes • Control
Rhus coriaria	• CS for 20, 40 and 60 days • Soaking in hot water (100°C) for 2 minutes • Immersion in concentrate (98%) H_2SO_4 for 30 and 60 minutes + 30-day CS • Control

The seeds were stratified by putting layers of moistened sand and seeds on top of each other. Since there was a risk for some of the seeds to be mixed with the sand because of their small size, linen cloth was placed between the sand and the seeds. The mean temperature of the room where cold stratification was applied on the seeds was +5°C. The moisture of the sand and the seeds were checked continuously against drying, heating and poor aeration. The medium was moistened so that the seeds did not become moldy. The moisture of the sand was measured by using a moisture tester.

Seeds were sown in polyethylene pots under greenhouse conditions and in seedbeds under open field conditions in the spring (March) of 2004. Polyethylene pots were filled with growing medium composed of forest soil, creek sand and manure (1:1:1). The experimental design was a randomized complete block with three replications (30 seeds

for each replication) for every treatment. The number of germinated seeds were counted every day, but recorded for 7th, 10th, 14th.and 21st days and in every week (7 days) after the 21st-day counting. Germination percentage and rate were determined according to each pretreatment and filled seed ratios were used to determine germination percentages. The below formula was used when determining germination rate (Pieper, 1952):

$$GR = \frac{(n1 \times t1) + (n2 \times t2) + (n3 \times t3) + (ni \times ti)}{T}$$

GR: Germination rate,
n: Number of days for each counting of germinated seeds.
t: Number of germinated seeds In each counting day.
T: Total number of germinated seeds.

The experiment approximately lasted for 90 days when it was observed that the seeds stopped germinating. Data from the treatments was analyzed by the SAS and SPSS statistical programmers. The ANOVA and Duncan tests were used to compare treatment groups whether they showed any statistically significance differences which was set at α=0.05. Approximate account of Satterthwaite was used to compute the differentials denominator degree of freedom to test greenhouse conditions and open field condition (Satterthwaite, 1946; Milliken and Johnson, 1984).

Results and discussion

Results showed that the seeds of *A. andrachne*, *C. creticus*, *C. armena*, *C. coggyria*, *C. numullaria*, *E. angustifolia*, *J. fruticans*, *P. spina-christii* and *R. coriaria* germinated under both greenhouse and open field conditions. All findings and discussions on germination rates and percentages of each species were evaluated and are summarized below:

Arbutus andrachne

The highest germination percentage for this species was 15.52% under open field conditions, which resulted from the treatments of 20-day cold stratification (CS) and submersion in 250 mgL^{-1} GA_3 for 20 minutes. The former treatment may therefore be preferred since it is cheaper than the latter. With regards to the rate of germination, the best result (23 days) was found when 40-day CS was applied under the greenhouse conditions (table 2). CS treatment was successful in breaking dormancy of *Arbutus* seeds as reported by Roy (1974) and Huxley *et al.* (1992). Karam and Al-Salem (2001) stated that at least 70-90 days of stratification was needed to overcome dormancy in *A. andrachne* seeds while Tilki (2004) reported that *A. unedo* seeds required 9 weeks of stratification.

It was observed that *A. andrachne* seeds were germinating while the stratification treatment was continuing. This may explain why, in general, the germination percentage of this species was lower than in some previous studies (Roy 1974; Huxley *et al.*, 1992; Karam and Al-Salem, 2001; Tilki, 2004). In addition, we found that better outcomes of germination percentages were recorded for open field conditions while germination rates were higher under greenhouse conditions.

Cistus creticus

Compared with other pretreatments the analyses showed that the soaking in hot water (100°C) resulted in better germination percentages than all others. Soaking in 100°C water for one minute gave the best germination percentages under green house conditions with 66.98%, while the lowest percentage was recorded for the pretreatments of CS of both 20 and 40-days with 4.42% and 5.44%, respectively (table 2). Similar findings were also reported by Pela *et al.* (2000) who stated that soaking of *Cistus* seeds in boiling water for 35 seconds or 1 minute showed the highest percentages of germination by 96% or 74% within 4 days. Pereirea *et al.* (1993) reported that germination increased with temperature. A maximum was reached at 90°C, decreasing very quickly thereafter, with no germination at 130°C or high temperatures.

Soaking in 100°C water for one minute under open field conditions resulted in the best germination rate (20 days) compared with the longest (51 days) for the control treatment. It was also observed that *C. creticus* seeds had started to germinate after two weeks of CS period.

It was reported that dry-heat treatments at 100°C for 5 minutes broke dormancy seeds of *C. clusii, C. monspeliensis* and *C. salvifolius* (Nadal *et al.*, 2002). All those reports and the results of this study showed that heating to 100°C treatment affected germination of *C. creticus* seeds positively. It was also found that *C. creticus* seeds germinated better in greenhouse than open field conditions. Collectively the results have indicated that seeds of *C. creticus*, like other *Cistaceae* species, show coat-imposed dormancy associated mainly with hardness. The hard seed-coat provides an effective barrier to water uptake that can be overcame by hot-water treatments.

Colutea armena

It was estimated that approximately 45% of the seeds were empty because of insect damage. Consequently these seeds were cleaned and disinfected before storage and sowing. The highest germination percentage of 91.25% was obtained from the seeds that were cold stratified for 20 days and sown under the open field conditions. The seeds soaked in 100°C water and then allowed to cool for 24 hours in the same water had lower germination percentages (21.85% in greenhouse and 29.76% under open field) than seeds soaked in tap water for 24 hours (43.63% in greenhouse and 55.54% under open field) (table 2). Similar findings was reported by Andrade (1983) who stated that soaking *C. arborescens* seeds initially in 100°C water and allowing them to remain in that water for 24 hours resulted in good seed germination percentage.

While soaking in tap water for 24 hours and sowing under the open field conditions resulted in the best germination rate (26 days), it was the longest (46 days) for the treatment, in which seeds were soaked in 100°C water and then allowed to cool for 24 hours in that water (table 2).

According to Pijut's (2004) study, some *Colutea* spp. seeds did not germinate easily unless the impermeable seed coat was ruptured by mechanical or chemical scarification. *C. armena* seeds used in this study were not scarified. Therefore, it may be true for this study that the treatments used could be insufficient to remove hard seed coat of *C. armena* because of the low germination percentages in general, except the seeds that were cold

Table 2. Germination percentages and rates achieved under greenhouse (G) and open field (OF) conditions for *Arbutus andrachne, Cistus creticus* and *Colutea armena* seeds.

Pretreatments	F-Ratio	GP (%)	F-Ratio	GR (day)
Arbutus andrachne				
Control (G)		1.07		44
10 min. in 250 mgL-1 GA_3 (G)	2.52**	1.07	13.33**	34
60-day CS (OF)		1.30		39
60-day CS (G)		2.17		42
10 min. in 250 mgL-1 GA_3 (OF)		3.24		48
Control (OF)		4.52		45
20 min. in 250 mgL-1 GA_3 (G)		5.40		30
40-day CS (OF)		5.82		43
20-day CS (G)		6.47		31
40-day CS (G)		10.77		23
20 min. in 250 mgL-1 GA_3 (OF)		15.52		42
20-day CS (OF)		15.52		39
Cistus creticus				
40-day CS (OF)	5.37*	4.42a	2.54	26
60-day CS (OF)	5.36**	5.44a		30
20-day CS (OF)		6.12ab		30
Control (G)		7.94ab		51
Soaking in water 180 min. at 50°C + 30-day CS (OF)		9.18ab		30
Soaking in water 180 min. at 50°C + 30-day CS (G)		10.20ab		47
40-day CS (G)		18.14ab		29
20-day CS (G)		23.81ab		25
60-day CS (G)		24.94ab		26
Control (OF)		27.55abc		31
Soaking in water 35 sec. at 100°C (OF)		29.25abc		28
Soaking in water 1 min. at 100°C (OF)		44.89abc		20
Soaking in water 35 sec. at 100°C (G)		47.62bc		39
Soaking in water 1 min. at 100°C (G)		66.89c		33
Colutea armena				
Soaking in 100°C water and then allowed to cool for 24 hours (G)		21.85		46
60-day CS (OF)		27.80		46
40-day CS (OF)	1.33	29.76	2.84	32
Soaking in 100°C water and then allowed to cool for 24 hours (OF)		29.76		41
Control (OF)		39.70		42
Control (G)		41.67		37
60-day CS (G)		43.63		30
Soaking in tap water for 24 hours (G)		43.63		32
20-day CS (G)		45.65		28
40-day CS (G)		55.54		28
Soaking in tap water for 24 hours (OF)		55.54		26
20-day CS (OF)		91.25		39

*Variation Source (VS): Greenhouse (Treatment), significantly different at α=0.05; **VS: Greenhouse*Open Field (Treatment), significantly different at α=0.05.

stratified for 20 days and sown under the open field conditions, resulting in 91.25%. It was also observed that some of *C. armena* seeds started to germinate by the 20th day while they were still in the CS medium, thereby decreasing the expected germination percentages.

Cotinus coggyria

Analyses showed that the pretreatments used in this study affected both seed germination percentage and rate in greenhouse conditions significantly, while they were not effective for seeds sown under the open field conditions. Increasing the duration of CS resulted in a significant increase in germination percentages of 30.61%, 39.68% and 44.22% for 20, 40 or 60 days, respectively. In contrast increasing the duration of immersion in H_2SO_4 resulted in decreasing germination percentages viz. 87.30%, 58.96% and 54.42% for 20, 50 and 80 minutes, respectively, followed by 60 days of CS. The treatment of immersion seeds in H_2SO_4 for 20 minutes with 60 days of CS gave the best results of germination percentages under both greenhouse (87.30%) and open field (38.55%) conditions (table 3). The best germination rate (16 days) was obtained from seeds soaked in H_2SO_4 for 80 minutes with 60-day CS under greenhouse conditions (table 3).

Previous studies (Dirr and Heuser, 1987; Takos and Efthimiou, 2002; Piotto *et al.*, 2003) that also used H_2SO_4 application and the combinations of CS and immersion in H_2SO_4 reported successful outcomes to overcome dormancy of *Cotinus coggyria* seeds. In general, the present results for *Cotinus coggyria* seeds were parallel to the above studies' findings since immersing in H_2SO_4 followed by cold stratification resulted in early, uniform and high germination percentage in greenhouse conditions.

Cotoneaster numullaria

The highest germination percentage of 53.67% was obtained from *C. numullaria* seeds that were soaked in H_2SO_4 for 90 minutes with 60-days CS (table 3). Using H_2SO_4 as a pretreatment to overcome dormancy of *Cotoneaster* spp. seeds was applied in similar studies done previously. For example, Kaminski (1985) reported that scarified seeds (by immersing in H_2SO_4 for 30-45 minutes) of *C. divaricata* followed by 180-day CS increased the germination percentage up to 80%. In addition, Meyer (1988) found out that there was no seed germination occurring before 58 days at any temperature tested. The present study showed that the durations lower than 60-days CS were not effective on germination of *C. numullaria* seeds, supporting the findings reported by Meyer (1988). In this study, it was interesting to note that longer durations of CS (more than 60-days) combined with immersing in H_2SO_4 caused an increase in germination percentage, indicating that *Cotoneaster* seeds exhibited double dormancy most probably due to their hard, impermeable seed coats and the physiological condition of their embryos.

The best germination rate (19 days), on the other hand, was determined from seeds soaked in H_2SO_4 for 30 minutes with 60-day CS (table 3).

Elaeagnus angustifolia

Germination percentages of *E.angustifolia* seeds soaked in running water at 15°C for 10 days followed by 30-days CS showed significant differences compared with control

Table 3. Germination percentages and rates achieved under greenhouse (G) and open field (OF) conditions for *Cotinus coggyria, Cotoneaster numullaria* and *Elaeagnus angustifolia* seeds.

Pretreatments	F-Ratio	GP (%)	F-Ratio	GR (day)
Cotinus coggyria				
40-day CS (OF)	14.56*	9.07a	14.50*	38cde
60-day CS (OF)	13.51**	15.31a	7.75**	43d
50 min. in H_2SO_4+ 60-day CS (OF)	4.32***	15.87a		38cde
Control (OF)		15.87a		41de
20-day CS (OF)		19.27ab		42de
Control (G)		23.81abc		53e
80 min. in H_2SO_4+ 60-day CS (OF)		29.48abc		40de
20-day CS (G)		30.61abc		33bc
20 min. in H_2SO_4+ 60-day CS (OF)		38.55bcd		37cd
40-day CS (G)		39.68bcd		28b
60-day CS (G)		44.22de		21a
50 min. in H_2SO_4+ 60-day CS (G)		54.42e		18a
80 min. in H_2SO_4+ 60-day CS (G)		58.96e		16a
20 min. in H_2SO_4+ 60-day CS (G)		87.30f		19a
Cotoneaster numullaria				
Control (G)		0.00a		-
40-day CS (G)		0.00a		-
60-day CS (OF)		0.00		-
Control (OF)	10.52*	1.24	5.60	25
20-day CS (OF)		1.24		30
40-day CS (OF)		1.24		25
60-day CS (G)		1.24a		30
20-day CS (G)		2.51a		24
90 min. in H_2SO_4+ 60-day CS (OF)		3.75		25
30 min. in H_2SO_4+ 60-day CS (OF)		7.49		28
60 min. in H_2SO_4+ 60-day CS (OF)		10.00		26
60 min. in H_2SO_4+ 60-day CS (G)		14.98a		21
30 min. in H_2SO_4+ 60-day CS (G)		17.49a		19
90 min. in H_2SO_4+ 60-day CS (G)		53.67b		20
Elaeagnus angustifolia				
Snipping off 2 mm (both ends) + soaking in water for 7 days (OF)		18.74a		42e
20-day CS (OF)	4.30*	21.42ab	34.98*	42e
Snipping off 2 mm (both ends) + soaking in water for 7 days (G)	8.27***	22.76ab	5.87***	30c
40-day CS (OF)		25.44abc		36d
60-day CS (OF)		26.77abcd		36d
Control (OF)		28.11abcd		42e
40-day CS (G)		41.49abcde		26bc
20-day CS (G)		42.84abcde		30cd
60-day CS (G)		49.53bcde		19a
Soaking in running water at 15°C for 10 days + 30-day CS (OF)		54.89cde		39e
Control (G)		56.22de		35d
Soaking in running water at 15°C for 10 days + 30-day CS (G)		64.26e		23ab

*VS: Greenhouse (Treatment), significantly different at α=0.05; **VS: Greenhouse*Open Field (Field), significantly different at α= 0.05; ***VS: Greenhouse*Open Field (Treatment), significantly different at α= 0.05.

sowings and other treatments. The best germination percentages, when this treatment was applied, were 64.26% and 54.89% under both greenhouse and open field conditions, respectively (table 3). It was observed that *E. angustifolia* seeds were moulded while they were in the stratification medium, but they were not moulded when maintained under running water at 15°C for 10 days. Fidelibus and MacAller (1993) stated, as ascribed to Lippitt (1992), that maintaining seeds in running water for 2 days reduced the levels of pathogenic fungi to a similar extent as chemical sterilizers without deleteriously effecting seed viability.

Olson and Barbour (2004) outlined, as ascribed to Carroll (1971), that stratification for less than 60 days was less effective than longer periods. Hamilton and Carpenter (1976) found that *E. umbellata* seeds stratified at 5°C from 14 to 42 days germinated less than 50% after 90 days at 25°C, whereas seeds stratified for 70 to 98 days germinated completely in 90 days. This, in turn, may indicate that using less than 60-days of CS in the present study might have caused the lower germination percentages reported by Carroll (1971) and Hamilton and Carpenter (1976).

Low germination percentages were obtained from the seeds that were exposed to the treatment of snipping off the seeds 2 mm at the radicle and cotyledon ends after soaking them in water for 7-days under both greenhouse (22.42%) and open field (18.74%) conditions (table 3). In contrast Belcher and Karrfalt (1979) reported that snipping off 2 mm at both the radicle and cotyledon ends after 7-day soaking in water resulted in 96%, whereas snipping off both ends only without soaking in water resulted in 100% germination.

Germination rates of *E. angustifolia* seeds sown in the greenhouse conditions showed significantly better results (germinated in fewer days) than those sown in the open field conditions. 60-day CS pretreatment gave the shortest germination rate (19 days) in greenhouse conditions.

Jasminum fruticans

It was found that cold stratification treatment was successful in overcoming seed dormancy of *J. fruticans*. Cold stratified seeds for 60 days showed the highest germination percentage (85.56%) in the greenhouse (table 4). The best germination rate (19 days) was also obtained from the seeds that were cold stratified for 60 days. When germination rates of *J. fruticans* seeds were considered it could be said that increasing of duration of stratification improved germination rates under both greenhouse conditions and open field conditions.

Paliurus spina-christii

Pretreatments that include immersing in H_2SO_4 significantly improved germination percentages of the seeds of *Paliurus spina-christii* when compared with cold stratification (table 4). Takos *et al.* (2001) reported that increasing the duration of soaking in H_2SO_4 caused an increase in germination percentages of the seeds. In this study, soaking in H_2SO_4 for 40 minutes showed the best germination percentages under both greenhouse and open field conditions with 65.05% and 54.99%, respectively.

Table 4. Germination percentages and rates achieved under greenhouse (G) and open field (OF) conditions for *Jasminum fruticans, Paliurus spina-christii* and *Rhus coriaria* seeds.

Pretreatments	F-Ratio	GP (%)	F-Ratio	GR (day)
Jasminum fruticans				
Soaking in water at 100°C for 30 sec. (G)		0.00a		0.00a
10 min. H_2SO_4 (G)		0.00a		0.00a
Soaking in water at 100°C for 30 sec. (OF)		0.00a		0.00a
10 min. H_2SO_4 (OF)		0.00a		0.00a
Control (OF)	32.73*	0.00a		0.00a
Control (G)	28.01**	4.44a	260.76***	33d
20-day CS (G)	5.79***	25.56b		24c
40-day CS (G)		38.89bc		21bc
60-day CS (OF)		43.33c		36de
40-day CS (OF)		51.11c		40ef
20-day CS (OF)		53.33c		41f
60-day CS (G)		85.56d		19b
Paliurus spina-christii				
60-day CS (OF)		3.37a		41
40-day CS (OF)		5.61a		51
Control (OF)		7.85a		48
20-day CS (OF)		8.99a		29
Control (G)	19.91*	8.98a	0.17	35
120 min. in H_2SO_4 (OF)	36.45**	10.10a	1.31	48
40-day CS (G)	43.81***	11.22a	1.18	33
60-day CS (G)		13.47a		30
20-day CS (G)		13.47a		33
120 min. in H_2SO_4 (G)		32.55b		33
80 min. in H_2SO_4 (OF)		49.38c		43
40 min. in H_2SO_4 (OF)		54.99c		43
80 min. in H_2SO_4 (G)		62.85c		31
40 min. in H_2SO_4 (G)		65.05c		31
Rhus coriaria				
60-day CS (G)		0.00a		00
60-day CS (OF)		0.00a		00+
40-day CS (OF)		1.03a		73+
20-day CS (OF)	6.67*	1.03a	17.35*	45+
Control (G)	16.78**	1.37a	10.61**	82+
Control (OF)	12.98***	2.06a	4.37***	27a
40-day CS (G)		5.49a	4.11****	42b
20-day CS (G)		8.23a		43b
Soaking in water at 100°C for 2 min. (OF)		12.35ab		70e
60 min. H_2SO_4+ 30-day CS (G)		16.46ab		21a
Soaking in water at 100°C for 2 min. (G)		16.46ab		54c
30 min. in H_2SO_4+ 30-day CS (OF)		27.78bc		41b
60 min. in H_2SO_4+ 60-day CS (OF)		28.80bc		41b
60 min. in H_2SO_4+ 30-day CS (OF)		33.95c		39b
30 min. in H_2SO_4+ 30-day CS (G)		34.29c		24a
60 min. in H_2SO_4+ 60-day CS (G)		35.67c		24a
30 min. in H_2SO_4+ 60-day CS (G)		39.78cd		17a
30 min. in H_2SO_4+ 60-day CS (OF)		54.53d		41b

*VS: Greenhouse (Treatment), significantly different at α=0.05; **VS: Open Field (Treatment), significantly different at α= 0.05; ***VS: Greenhouse*Open Field (Treatment), significantly different at α=0.05; ****VS: Greenhouse*Open Field (Field), significantly different at α=0.05; + Analysis were not made because of the differentials between replications.

The best germination rate (31 days) was also found for both treatments of soaking in H_2SO_4 for 40 and 80 minutes in greenhouse conditions.

Takos *et al.* (2001) also reported that increasing CS duration resulted in a significant increase in germination percentage of *P. spina-christii* seeds. They suggested that stratification periods of 60, 90 and 120 days for good germination percentages of the *P. spina-christii* seeds. In contrast, in this study, the 60-days CS treatments used in both greenhouse and open field conditions showed lower germination percentages, 13.47% and 3.37%, respectively. However, CS treatments lower than 60-days resulted in very low germination percentages thereby coinciding with the suggestions of Takos *et al.* (2001) for longer CS periods.

Rhus coriaria

Several combinations of soaking in H_2SO_4 with CS treatments gave significantly better results in breaking *R. coriaria* seed dormancy than all other treatments (table 4). Soaking in H_2SO_4 for 30 minutes with CS for 60 days increased germination percentages of *R. coriaria* seeds under both greenhouse and open field conditions up to 39.78% and 54.53%, respectively (table 4). The degree of seedcoat hardness and embryo dormancy varies within and among seedlots for most species (Hartmann *et al.*, 1997). This is true for *Rhus* species consequently treatments to overcome seed dormancy vary among *Rhus* species (Li *et al.*, 1999b). In addition, previous studies showed that the *R. coriaria* seeds have double dormancy; hardcoated seed and additional embryonic dormancy (Doussi and Thanos, 1994; Takos and Efthimiou, 2002).

In the present study it was found that scarification by immersing in H_2SO_4 solution followed by stratification treatments resulted in better germination percentages than using only scarification by immersing in H_2SO_4 or using hot water alone. Similar results were reported by Heit (1967) in that both scarification and stratification pretreatments were necessary for optimum seed germination percentages of this species. Li *et al.* 1999a also reported that concentrated H_2SO_4 broke seed dormancy in *R. aromatica*, whereas soaking in hot water broke dormancy in seeds of *R. glabra.*

CS and soaking in water at 100°C for 2 minutes treatments did not improve germination percentages of the seeds; contradicting the findings of Li *et al.* (1999a) who found that soaking in hot water treatments were effective on breaking seed dormancy of some *Rhus* species.

Conclusions

Overall, it can be said that there were cumulative effects of applying various combinations of immersion in H_2SO_4 solution followed by CS treatments on the germination percentages and rates of the *C. coggyria*, *C. numullaria*, *P. spina-christii* and *R. coriaria* seeds used in this study. The results indicated that using CS treatment alone can be sufficient to break the dormancy for seeds of *J. fruticans.* In addition to cold stratification treatments, soaking in running water can affect the germination percentages of *E. angustifolia* seeds positively. Germination percentage of *C. creticus* seeds can be increased through soaking them in hot water (100°C). The results also showed that the durations of CS or

scarification are insufficient to overcome dormancy of *A. andrachne* and *C. armena* seeds. For that reason, the durations of CS or scarification need to be determined according to characteristics of the species. It can also be concluded that the greenhouse conditions were more effective on germination percentages and rates of all the species used in this study over open field conditions.

Acknowledgements

The authors would like to thank Dr. Mehmet Ozalp for his input in the manuscript preparation.

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