

Effects of some pretreatments on germination of bladder-senna (*Colutea armena* Boiss. and Huet.) and smoke-tree (*Cotinus coggygia* Scop.) seeds

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Abstract: This study was carried out to determine effects of pre-treatments including floating in hot water (100°C) followed by continual cooling for 24 hr in the same water, floating in tap water for 24 hr, submersion in concentrate (98%) sulphuric acid (H₂SO₄) and cold stratification for different durations (20, 40 and 60 days) and their combinations on seed germination and to investigate how to overcome dormancy of seeds of *Colutea armena* Boiss. and Huet. and *Cotinus coggygia* Scop. The seeds were sown in polyethylene pots in the greenhouse and on seedbeds under open field conditions. The statistical design was a randomized complete block design with three replications. The highest germination percentage (77.19%), the best germination rate (16 days) and the highest growth rate (69.01%) were obtained from *Colutea armena* seeds that were submersed in sulphuric acid for 30 min and sown in the greenhouse. The pre-treatment of submersion in sulphuric acid for 20 min with cold stratification for 60 days gave the highest germination percentage (82.77%) and the highest growth rate (79.37%) in the greenhouse for *Cotinus coggygia* seeds. On the other hand, the best germination rate (9 days) was obtained from smoketree seeds that were cold stratified 60 days and sown under open field conditions. It can be stated that there is an affirmative effect of the greenhouse condition on germination percentage and growth rate values of the seeds used in this study.

Key words: *Colutea armena*, *Cotinus coggygia*, Seed dormancy, Pretreatments, Germination

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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 (Pritchett and Fisher, 1987; Balci, 1996). *Colutea armena* Boiss. and Huet. and *Cotinus coggygia* Scop. growing in steep and rocky landscapes are drought-tolerant plants that are important in preventing soil erosion. These species are also important as ornamental plants and used as an alternative income source for the local people since various parts (e.g. fruits, flowers and roots) of these plants are commercially important (Rudolf, 1974; Dirr, 1990; Urgenc, 1986; Krussmann, 1984; Gilman and Watson, 2003; Olmez *et al.*, 2007a).

Seeds of many woody plant species cannot germinate even if they are sown under proper moisture, oxygen and soil conditions on that year (Urgenc and Cepel, 2001). This problem is called seed dormancy and its causes are hard and impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover (ISTA, 1966, 1993). Baskin and Baskin (2004), have classified the types of seed dormancy as physiological, morphological, morpho-physiological, physical and combined dormancies. There is great deal of variation in germination ability of seeds even within the same species. Poulsen (1996) reported by

referring to Wolf and Kamondo (1993) that dormancy among and within seed-lots of the same species varies with provenance, crop year and individual trees.

According to some researchers, there are various germination obstacles in *Colutea* sp (Dirr and Heuser, 1987; Dirr, 1990; Olmez *et al.*, 2006; Olmez *et al.*, 2007b; Olmez *et al.*, 2007c) and *Cotinus coggygia* seeds (Tako and Efthimiou, 2002; Piotto *et al.*, 2003; Olmez *et al.*, 2007a,c) resulting in propagation difficulties. There have been few studies to determine different methods and techniques to overcome seed dormancy in bladder-senna and smoketree species. Generally pre-treatments such as floating in hot water, mechanical and chemical scarification and hot aeration are used for seed coat dormancy while the cold and warm stratifications are usually applied to dormancy caused by restrictions at the embryo level (Landis *et al.*, 1996; Tilki and Dirik, 2007). Among these methods and techniques, especially cold stratification, submersion in concentrate H₂SO₄, steeping seeds in hot water (88-100°C) followed by 24 hr chilling are well-known ones used to increase germination percentage of *Colutea* L. and *Cotinus coggygia* seeds (Dirr and Heuser, 1987; Bird, 1990; Dirr, 1990; Tako and Efthimiou, 2002; Piotto *et al.*, 2003; Olmez *et al.*, 2007a, b, c; Cicek and Tilki, 2007).

The aim of this study was to examine the influence of some pre-treatments [floating in hot water (100°C) followed by continual cooling for 24 hr in the same water, floating in tap water for 24 hr, submersion in concentrate (98%) sulphuric acid (H₂SO₄) and cold

stratification] on dormancy of *Colutea armena* and *Cotinus coggygia* seeds.

Materials and Methods

Ripe fruits of the species were collected from wild in the Artvin region, located in the Northeastern part of Turkey, between the altitudes of 200 and 1200 m, in August and September 2004. The seeds were separated from the fruit material, rinsed in tap water, dried in the shade, and stored at $4\pm1^\circ\text{C}$ in plastic bags after ratios of filled seed were determined.

The pre-treatments were listed in Table 1 for both two species in the study. These pre-treatments were applied to determine their effects on germination percentage (GP), germination rate (GR) and growth rate (GrR) the number of seeds survived after germination) for each species.

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 mixing seeds with the sand due to their small size, linen cloth was placed between the sand and the seeds. The mean temperature of the room where cold stratification (CS) was applied was about $5\pm1^\circ\text{C}$. 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 seeds were sown in polyethylene pots in the greenhouse and in seedbeds under open field conditions in the spring (March) of 2005. 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 per replication) for each treatment. The number of germinated seeds was counted every day, but recorded at 7th, 10th, 14th and 21st days and recording counted weekly (7 days) after the 21st day for about 90 days counting. Germination percentage, germination rate and growth rate were determined according to each pre-treatment and filled seed ratios were used to determine germination percentages. The following formula was used for determining germination rate (Pieper, 1952):

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

Where:

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 lasted for approximately 90 days and finalized when the observation showed that the seeds stopped germinating. Data from the treatments was analyzed by the SAS and SPSS version 11.5 statistical programs. ANOVA and Duncan's tests were used to compare pre-treatment groups to find out whether they showed any statistically significance differences at α 0.05.

Approximate account of Satterthwaite was used to compute the differentials denominator degree of freedom to test greenhouse and open field conditions (Satterthwaite, 1946; Milliken and Johnson, 1984). For evaluating data from greenhouse and open field observations and to compare greenhouse and open field, statistical model were used as below:

To analyze greenhouse conditions and open field,

$$y_{ijk} = \mu + r_i + \tau_j + rt_{ij} + e_{ijk} \text{ Models were used.}$$

y_{ijk} = Observed merit of at k . seed of j . pre-treatment of i . replication;

μ = General average;

r_i = Random effect of i . replication, $E(r_i) = 0$, $\text{Var}(r_i) = S_r^2$;

τ_j = Constant effect of j . pre-treatment, $\sum_{j=1}^n \tau_j = 0$;

rt_{ij} = Interaction between i . replication and j .

pre-treatment, $E(rt_{ij}) = 0$, $\text{Var}(rt_{ij}) = S_{rt}^2$;

e_{ijk} = Coincidental error, $E(e_{ijk}) = 0$, $\text{Var}(e_{ijk}) = S_e^2$;

To compare greenhouse conditions and open field;

$$y_{ijkl} = \mu + \alpha_k + r(a)_{i(k)} + \tau_j + \alpha\tau_{kj} + rt(a)_{ij(k)} + e_{ijkl} \text{ Models were used.}$$

y_{ijkl} = Observed merit of at l . seed of j . pre-treatment of i . replication of k . field ;

α_k = Constant effect of k . field; $E(a_k) = 0$, $\text{Var}(a_k) = S_a^2$;

$r(a)_{i(k)}$ = Random effect of i . replication at k . field; $E(r(a)_{i(k)}) = 0$,

$\text{Var}(r(a)_{i(k)}) = S_{r(a)}^2$;

τ_j = Random effect of j . pre-treatment, $\sum_{j=1}^n \tau_j = 0$

$\alpha\tau_{kj}$ = Interaction between k . field and j . pre-treatment, $\sum_{k=1}^2 \sum_{j=1}^n \alpha\tau_{kj} = 0$

$rt(a)_{ij(k)}$ = Interaction between i . replication at k . field and j . pre-treatment, $E(rt(a)_{ij(k)}) = 0$,

$\text{Var}(rt(a)_{ij(k)}) = S_{rt(a)}^2$;

e_{ijkl} = Random error, $E(e_{ijkl}) = 0$, $\text{Var}(e_{ijkl}) = S_e^2$

Results and Discussion

Results showed that the seeds of *Colutea armena* and *Cotinus coggygia* germinated both in the greenhouse and under open field conditions. All findings and discussions on GP, GR and GrR of each species were evaluated and are summarized below:

***Colutea armena*:** It was estimated that approximately 50% of the seeds were empty because of insect damage. Consequently the

Table - 1: Pre-treatments for overcoming the seed dormancy

Species	Pretreatments
<i>Colutea armena</i>	<ul style="list-style-type: none"> • Cold stratification (CS) for 20, 40 and 60 days • Floating in hot water (100°C) followed by continual cooling for 24 hr in the same water • Floating in tap water for 24 hr • Submersion in concentrate H_2SO_4 for 30 min • Control (no treatment)
<i>Cotinus coggygia</i>	<ul style="list-style-type: none"> • Cold stratification (CS) for 20, 40 and 60 days • Submersion in concentrate H_2SO_4 for 20, 50 and 80 min + 60-day CS • Control (no treatment)

Table - 2: Germination percentage, germination rate and growth rate achieved under greenhouse (G) and open field (OF) conditions for *Colutea armena* seeds

Pretreatments	F-ratio	GP (%)	F-ratio	GR (day)	F-ratio	GrR (%)
20-day CS (OF)		10.53 ^a		42		3.51 ^a
Control (OF)	3.52*	11.70 ^a	5.37*	39	3.85**	4.68 ^a
60-day CS (OF)	9.15**	14.04 ^a		39	8.36***	4.68 ^a
Floating in tap water for 24 hr (OF)	7.58***	15.20 ^a		30		5.85 ^{ab}
40-day CS (OF)		16.37 ^a		25		11.70 ^{abc}
20-day CS (G)		30.41 ^{ab}		43 ^{bc}		28.07 ^{bcd}
Floating in tap water for 24 hr (G)		32.75 ^{ab}		40 ^{bc}		31.58 ^{cd}
Floating in 100°C followed by continual for 24 hr in the same water (OF)		35.09 ^{ab}		18		16.37 ^{abc}
Control (G)		42.11 ^b		49 ^c		39.77 ^d
40-day CS (G)		43.27 ^b		43 ^{bc}		43.27 ^d
60-day CS (G)		47.95 ^{bc}		40 ^{bc}		42.11 ^d
Floating in 100°C followed by continual for 24 hr in the same water (G)		49.12 ^{bc}		33 ^b		49.12 ^{de}
Submersion in H_2SO_4 for 30 min (OF)		69.01 ^{cd}		20		28.07 ^{bcd}
Submersion in H_2SO_4 for 30 min (G)		77.19 ^d		16 ^a		69.01 ^e

*VS = Greenhouse (treatment), significantly different at $p \leq 0.05$, **VS = Open field (treatment), significantly different at $p \leq 0.05$,

***VS = Greenhouse, * = Open field (treatment), significantly different at $p \leq 0.05$

filled seeds were cleaned and disinfected before storage and sowing. The highest GP of 77.19%, GrR of 69.01% and the best GR of 16 days were obtained from the seeds that were immersed in sulphuric acid for 30 min and sown in the greenhouse (Table 2). According to Dirr (1990), the seeds of some *Colutea* species did not germinate easily unless the impermeable seed coat was ruptured mechanical and chemical scarification. Olmez *et al.* (2007c) implied that CS pre-treatment alone could not be sufficient to remove hard seed coat of *C. armena* and suggested chemical scarification. Dirr (1990) also stated that soaking *C. arborescens* seeds in sulphuric acid for 30 to 60 min resulted in good germination. In addition, soaking *C. armena* seeds in concentrate sulphuric acid for 30 min before sowing in the laboratory conditions, resulted in good germination percentage (82.8%) (Olmez *et al.*, 2007b). Therefore, it may be true for our study that the CS pre-treatments used could be insufficient to remove hard seed coat of *C. armena*. While submersion in sulphuric acid for 30 min and sowing in the greenhouse resulted in the best germination rate (16 days), it was the slowest (49 days) for the control seeds (Table 2).

The highest GP value among the CS pre-treatments was 47.95% for the seeds that were cold stratified for 60 days. The hot water pre-treatment resulted in better GP (49.12%) compared to the tap water pre-treatment (32.75%) in the greenhouse (Table 2). These findings are supported by other similar studies done on *C. arborescens* seeds (Allue Andrade, 1983; Dirr and Heuser, 1987). Dirr (1990) stated that soaking *Colutea* L. seeds initially in hot water (88°C) and allowing them to remain in that water for 24 hr resulted in good GP values. In addition, Piotto *et al.* (2003) reported that scarified *Colutea* L. seeds followed by floating in hot water (80°C) gave high GP and a short time to maximum GR.

***Cotinus coggygia*:** Analyses showed that the pre-treatments used in this study affected seed GP, GrR and GR both in the greenhouse and under open field conditions significantly. Compared to open field conditions the analyses showed that the greenhouse conditions showed significant differences and gave the highest GP and GrR values for *C. coggygia* seeds (Table 3).



Table - 3: Germination percentage, germination rate and growth rate achieved under greenhouse (G) and open field (OF) conditions for *Cotinus coggygia* seeds

Pretreatments	F-ratio	GP (%)	F-ratio	GR (day)	F-ratio	GrR (%)
Control (OF)		0.00 ^a		0 ^a		0.00 ^a
40-day CS (OF)	14.09*	2.27 ^a	42.07*	19 ^{ade}	11.72*	1.13 ^a
20-day CS (OF)	2.01**	2.27 ^a	1.33**	++	3.00**	0.00 ^a
60-day CS (OF)	49.25***	4.54 ^{ab}	11.49***	9 ^{abc}	58.32***	0.00 ^a
Submersion in H ₂ SO ₄ for 20 min+60-day CS (OF)		9.07 ^{ab}		12 ^{abcd}		3.40 ^a
Submersion in H ₂ SO ₄ for 50 min+60-day CS (OF)		9.07 ^{ab}		11 ^{abcd}		1.13 ^a
Submersion in H ₂ SO ₄ for 80 min+60-day CS (OF)		12.47 ^{ab}		14 ^{bcd}		4.54 ^a
Control (G)		19.27 ^b		52 ^a		19.27 ^b
20-day CS (G)		52.15 ^c		37 ^f		47.62 ^c
60-day CS (G)		62.36 ^c		30 ^{ef}		62.36 ^d
40-day CS (G)		62.36 ^c		32 ^{ef}		61.22 ^d
Submersion in H ₂ SO ₄ for 50 min+60-day CS (G)		77.10 ^d		22 ^{de}		74.83 ^{de}
Submersion in H ₂ SO ₄ for 80 min+60-day CS (G)		77.10 ^d		24 ^{de}		74.83 ^{de}
Submersion in H ₂ SO ₄ for 20 min+60-day CS (G)		82.77 ^d		25 ^{def}		79.37 ^e

*VS = Greenhouse (treatment), significantly different at $p \leq 0.05$. **VS = Open field (treatment), significantly different at $p \leq 0.05$.

***VS = Greenhouse*Open field (treatment), significantly different at $p \leq 0.05$, * Analysis were not made because of the differentials between replications

Submersion in H₂SO₄ for 20 min with CS for 60 days gave the highest GP (82.77%) and GrR (79.37%) in the greenhouse. CS and submersion in sulphuric acid pre-treatments gave higher GP and GrR values in the greenhouse than open field conditions (Table 3).

Increasing the duration of CS resulted in an increase in GPs of 52.15%, 62.36% and 62.36% for 20, 40 or 60 days in the greenhouse, respectively (Table 3). Previous studies that used sulphuric acid application and the combinations of CS and immersion in sulphuric acid reported successful outcomes to overcome dormancy of *C. coggygia* seeds (Dirr and Heuser, 1987; Takos and Efthimiou 2002; Piotto et al., 2003; Olmez et al., 2007a). Takos and Efthimiou (2002) reported a higher germination (73%) in the laboratory than nursery conditions (19%) in *C. coggygia* seeds when submersion in H₂SO₄ for 30 min followed by CS for 60 days pre-treatment was used. In general, the present results for *C. coggygia* seeds were parallel to the above studies' findings since immersing in H₂SO₄ followed by CS resulted in early, uniform and high GP and GrR in the greenhouse.

Consequently, among all the pre-treatments applied to the *Colutea armena* seeds, submersion in sulphuric acid for 30 min in the greenhouse resulted in the highest GP (77.19%), GrR (69.01%) and the best GR (16 days). The results for indicate that the pre-treatment of submersion in sulphuric acid for 20 min with CS for 60 days could be preferably used to overcome germination dormancy of *Cotinus coggygia* seeds. It can be also stated that there is an

affirmative effect of the greenhouse condition on GP and GrR of the seeds used in this study.

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