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EFFECTS OF DIFFERENT PRETREATMENTS ON THE GERMINATION OF DIFFERENT WILD CHERRY (*PRUNUS AVIUM* L.) SEED SOURCES

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Abstract

Wild cherry (*Prunus avium* L.) is an important native hardwood species of Turkey with its high-valued wood. It is also listed as a 'noble hardwood' in Europe with strongly emphasized conservation, sustainable use and genetic potential. Wild cherry seeds are deeply dormant, and level of dormancy may vary significantly within the species. This causes substantial difficulties during seedling production. The effects of different artificial and natural pretreatments in seed germination of three Turkish wild cherry seed sources was examined. The effects of different 0.1 % citric acid soaking treatments prior to cold stratification on the seed germination of a separate seed source was also examined. Pretreatments made significant effects on speed of and cumulative seed germination with substantially different seed source responses. Successive periods of complex, warm and cold artificial stratification regimes rather than cold period alone as well as natural stratification substantially improved the dormancy breaking and germination of wild cherry seeds. Seeds from K. Ereğli were superior over those of the other seed sources in both speed and cumulative rate of germination. Seeds soaked in 0.1% citric acid for 48 hours followed by a 90-day cold stratification period enhanced germination significantly, yet longer acid exposures decreased seed germination substantially.

Introduction

The gap between Turkey's demand for and supply of quality hardwood has dramatically widened (Boydak & Dirik, 1998; Anon., 2001). Intensively growing fast-growing native hardwood tree species on selected forest and agricultural lands is recommended to narrow this gap (Boydak & Dirik, 1998; Anon., 2001). With its fast-growth, high-valued wood (Savill 1991; Eriksson 2001; Martinsson 2001), wildlife (Grisez 1974) and biodiversity values in forest ecosystems (Kobliha, 2002), wild cherry (*Prunus avium* L.) has recently drawn great attention in Turkish forestry (Yaman 2003, Eşen *et al*., 2005). Wild cherry is also designated as a 'noble hardwood' by the European Forest Genetic Resources Program, which encourages research on the species to develop strategies for the conservation and sustainable use (Eriksson 2001; Kobliha 2002).

Adequate seed germination is the key to successful tree establishment (Radosevich *et al*., 1997). Wild cherry seed has deep dormancy that requires a long, variable period of moist low temperatures, interrupted by shorter periods of moist warm temperatures to germinate (Suszka, 1962; Grisez, 1974; Ellis *et al*., 1985; Finch-Savage, 2001; Finch-Savage *et al*., 2002). Such broad variation in germination behavior results in major difficulties during seedling production of the species in Turkey. The objectives of this study were to determine the effects of different artificial and natural pretreatments on the dormancy and germination behaviors of the seeds of three different wild cherry seed sources (SSs), from the Western Black Sea Region of Turkey. Effects of soaking seeds in 0.1% citric acid solution for varying periods prior to a standard cold pretreatment on seed germination were also determined in this study. The objective of the study was to determine the pretreatment that would confer the greatest cumulative germination rate for wild cherry during future practices in nurseries and regeneration projects.

Materials and Methods

Mature fruits (i.e., after fruit colors turned from red to dark-red) of three different wild cherry seed sources (SSs) were collected from open-pollinated single mature trees (nearly 30-40 yrs old) growing in the western Black Sea Region of Turkey between July-August, 2003. Of the SSs, the Ereğli SS have mixed fruits from the entire Forest Management Unit of K. Ereğli. The pulps of fruits were cleaned manually, using sand and water. Extracted stones were then dried at room temperature for two days. All stones had been stored in a refrigerator at 3°C for 4-5 months until treatments began.

Stones (hereafter called seed unless otherwise stated) were first studied for various physical characteristics (Table 1). Seed moisture contents before the beginning of the pretreatments were calculated using International Seed Testing Association rules for seed testing (Anon., 1999). Mean seed diameter for each seed collection was assessed using 20 randomly sampled seeds. Four random samples of 100 seeds from each SS were used to determine the mean seed soundness i.e., stones with completely developed seeds (Grisez, 1974).

All of the seeds were soaked in dionized water for 24 hours. They were later mixed in 4:1 proportion with a moist peat moss medium (Grisez, 1974) for adequate aeration in large zipped plastic bags that permit gas exchange. Five different periods of cold or warm-and-cold stratifications adapted from Grisez (1974) and Hofman & Steiner (2005, pers. comm.) of the NFV, Germany and the check (no pretreatment at all) were used for this study (Table 2). For cold stratification (CS), the seed bags were stored at 3^{o}C (±2) in a cold room. For warm stratification (WS), seed bags were stored in a growth chamber (Nüve ID 501®, Nüve, Inc., Ankara, Turkey) at 20^{o}C (±0.5). All seed bags were regularly aerated and checked for moisture twice or three times a week. In addition, the seeds of a different SS (Günbaşı) were soaked in 0.1% citric acid solution for varying periods including one, two, three and four day(s) prior to a 90-day CS treatment (Jones 1963; Grisez 1974).

For natural stratification, the seeds were first sown in rows in nursery beds at the Düzce Forest Nursery (40°50'N, 31°10' E, 140 m. asl) at the beginning of November 2003. The nursery had sandy-clayey loamy soil with a pH of 7.2-7.5 in the rooting zone, a mean annual precipitation of 84 cm, a mean temperature of 13^{o}C (Anon., 1999, 2005). One hundred seeds were used in each of the four replications for each SS. The SSs were randomly assigned to rows. The seeds in the rows were covered using a 1:1:1 mix of peat moss, washed sand, and sheep manure. Germination percentage of each SS was assessed in the late May 2004.

Following the artificial pretreatment, the seeds of each SS and the check treatment (four replicates of 100 seed each) were placed in a moist sand medium in 18-cm glass Petri dishes. The dishes were then placed into the environment-controlled growth chamber (20±1^{o}C, Anon., 1999) for germination and kept moist during the germination test. All of the dishes were monitored for germination on days 4, 7, 10, 21, and finally day 28. The seeds with 5 mm long radicles were considered germinated (Anon., 1999). Cumulative germination percentage was calculated as the proportion of germinants on day 28 to the total number of sound seeds used, multiplied by 100.

Table 1. Source locations and various physical characteristics of wild cherry (*Prunus avium*) seeds in the western Black Sea Region of Turkey.

Seed source[1]	Elevation (m)	Harvest year	The weight per 1000 seeds (g)	Seed number per kg	Seed diameter (mm)	Moisture of air-dried seed (%)	Seed soundness (%)
Cumaova, Düzce, Bolu	150	2003	147	6,789	6.2	9	72
Karadeniz Ereğlisi, Zonguldak	-	2003	140	7,138	6.0	11	51
Pınarbaşı, Kastamonu	800	2003	199	5,034	6.8	13	43

[1]Word arrangement follows the Forest Management Unit, Forest Management Directorate, and Forest Management Regional Directorate of the seed sources

Table 2. Effects of different seed sources and artificial stratification methods on germination of wild cherry (*Prunus avium*) seeds.

Pretreatment[1,2]	Cumaova	Ereğli	Pınarbaşı
	Germination percentages (%) and Standard errors (±)		
90DCS	1.2 a[3] (±0.2)	1.5 c (±1.2)	0.3 b (±0.2)
15DWS + 90DCS	7.7 a (±1.8)	8.2 b (±1.1)	3.4 ab (±1.2)
120DCS	1.5 a (±0.5)	24.8 a (±4.1)	0.5 b (±0.3)
15DWS + 120DCS	2.2 a (±1.3)	pg	pg
20DWS + 15DCS + 15DWS + 85DCS	6.7 a (±3.1)	23.9 a (±4.3)	7.9 a (±1.8)
No stratification (the check)	0	0	0

[1]The seed-source x pretreatment interaction term was significant after ANOVA ($p \leq 0.05$)
[2]D: Days; C: Cold stratification; W: Warm stratification; pg: premature germination before the end of the stratification
[3] Treatments within the same column with different letters are significantly different ($p \leq 0.05$)

The experimental design for the natural and artificial stratification experiments were a randomized complete block design and completely randomized design with four replications, respectively. The statistical analysis (Analysis of variance, ANOVA) was used to test for main (SS and pretreatment) and SS X pretreatment interaction effect. The means were separated using the adjusted Tukey-Kramer Mean Comparison Test. *P*-values ≤ 0.05 were considered significant throughout the experiment. Arcsine transformations were carried out on all cumulative germination percentage data before analysis. The untransformed data were tabulated.

Results

Generally, there was not a large range in the physical seed characteristics among the SSs (Table 1). The mean air-dried pregermination moisture content of SSs ranged between 9-13%. The mean seed soundness was variable across the SSs: The Cumaova SS had a high percentage of stones with fully developed seed.

The SSs responded substantially different to different stratification treatments, with a significant SS X pretreatment interaction (results not shown). Seeds in the check showed no germination. Mean germination percentage was generally low across the SSs (Table 2).

Pretreatments did not significantly affect the mean cumulative germination percentage for the Cumaova SS (Table 2). There were yet significant germination differences among pretreatments for the other SSs. For the Ereğli SS, the 90-day cold stratification (DCS) was the poorest pretreatment for the mean cumulative seed germination. When this treatment was however preceded with one or two short warm period(s) (WS), mean cumulative percent germination significantly increased (>5-fold) for both of the Cumaova and Ereğli SSs. Both of the 90- and 120-DCS pretreatments were insufficient to break seed dormancy. There were large yet nonsignificant differences among the SSs for the natural stratification (fall sowing, Table 3). The percent germinations of the SSs in the most complex artificial pretreatment (20DWS + 15DCS +15DWS + 85DCS) and natural stratification experiments appeared to match (Tables 2, 3).

Table 3. The mean germination percentages of the seeds of various wild cherry seed sources in natural stratification (fall-sowing).

Seed source[1]	Germination percentages (%) and Standard Errors (±)
Ereğli	20.1 a[2] (±4.1)
Cumaova	9.1 a (±2.4)
Pınarbaşı	3.1 a (±1.0)

[1]Seed-source main effect was significant ($p \leq 0.05$)
[2]Means with the same letters are not significantly different ($p \leq 0.05$)

Table 4. Effects of 0.1 % Citric-acid-soaking + cold stratification combined treatment on germination of wild cherry seeds from Günbaşı.

Pretreatment[1]	Mean germination percentages (%) and Standard Errors (±)
48 h CiA + 90D C	27.0 a[2] (±4.2)
90D CS (the check)	14.0 ab (±2.4)
72 h CiA + 90D C	12.0 b (±1.6)
24 h CiA + 90D C	8.5 b (±1.5)
96 h CiA + 90D C	2.0 c (±1.2)

[1] D: Days; C: Cold stratification; CiA: 0.1% citric acid treatment
[2] Means with different letters are significantly different ($p \leq 0.05$).

Soaking of seeds in 0.1% Citric acid (CiA) for varying periods prior to the standard 90 DCS significantly affected germination percentages (Table 4). The seeds of the 48-hour CiA soaking treatment plus 90DCS had a greater mean percent cumulative germination than the standard pretreatment, yet with a nonsignificant difference. The 24-hour and prolonged (>48-hour) CiA exposures significantly reduced mean seed germination rate.

Pretreatments also substantially affected seed germination speed for different SSs (Fig. 1). Germination speed was sluggish by day 10 and did not culminate by week 3 in the 90DCS pretreatment. The Ereğli SS had mostly a faster germination rate, when compared to those of the others in the rest of pretreatments. Germination was consistently very poor in the 120DCS pretreatment for the Cumaova and Pınarbaşı SSs. All SSs exhibited a progressively increasing germination in the most complex pretreatment (Fig. 1).

Discussion

Seed moisture content can significantly affect germination rate in cherry species (Grisez, 1974). The mean moisture contents of wild cherry seeds in the present study did not vary substantially and were mostly above the critical levels (9-11%) reported for adequate germination of cherry species (Suszka *et al.*, 1996). Seed characteristics rarely vary with different seed sources for cherry species (Grisez, 1974). Similarly, no large differences in the physical seed characteristics studied in the present study were found among the three Turkish SSs. High variation in seed soundness that has been reported for wild cherry (Tukey, 1927, Grisez, 1974) was also observed in the present study. A negative relationship that appeared between seed size and germination for wild cherry in the present study was previously reported for *Prunus serotina* Ehrh., black cherry (Pitcher, 1984).

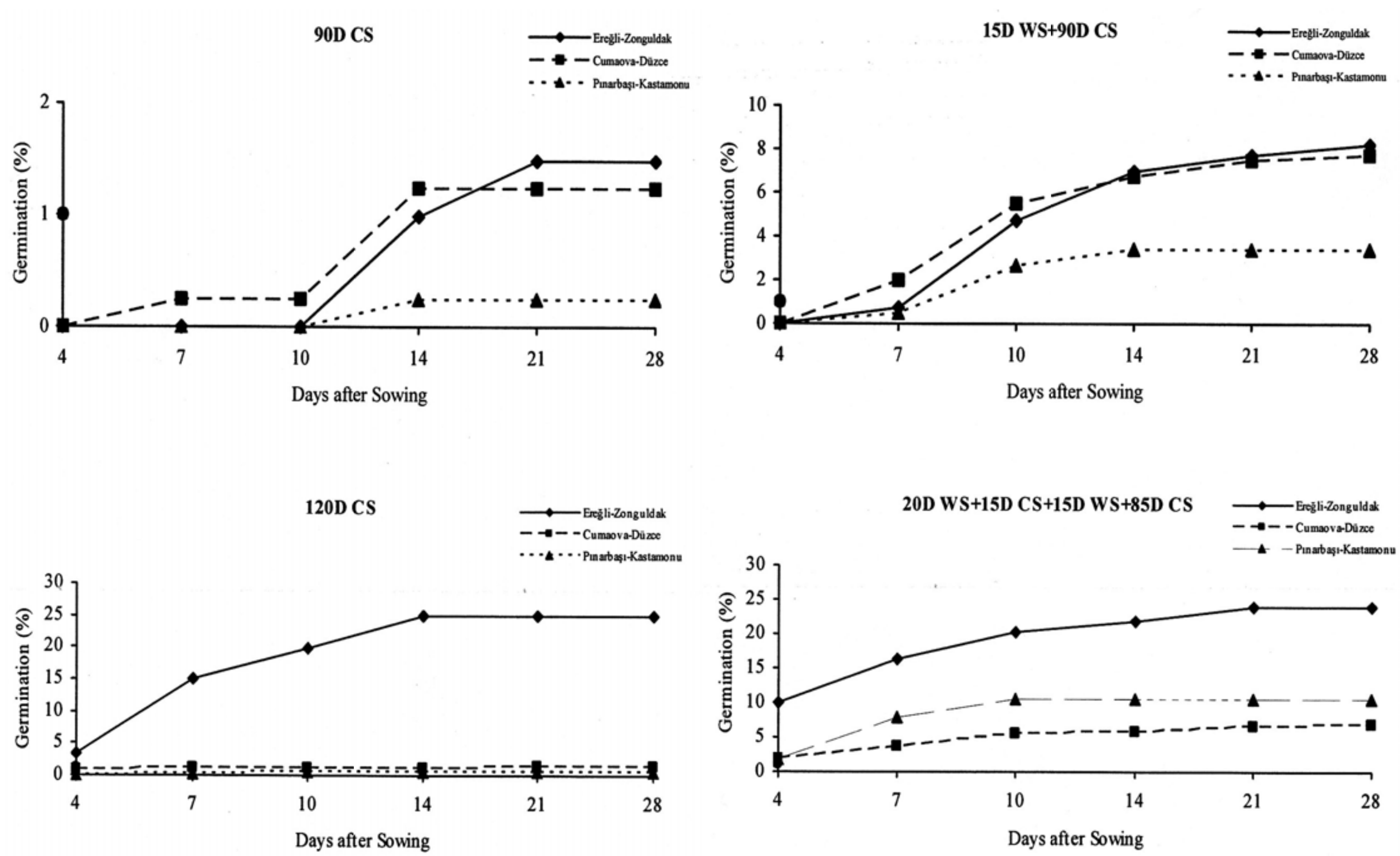

Fig. 1. Percent germination over time of the seeds of different wild cherry (*Prunus avium*) seed sources collected from the western Black Sea Region of Turkey under different artificial stratifications. Note the different scales of the Y-axes.

Cherry seeds have 'an embryo dormancy', which is generally corrected by 3-4 months of cold stratification (Grisez, 1974). Significantly improved mean cumulative seed germination in the present study by consecutive periods of warm and cold artificial stratification regimes and natural stratification confirmed the previous reports in wild cherry seed germination trials. These trials concluded that 'complex' stratification regimes with consecutive warm and cold periods allowed cherry seeds to continue their development and overcome its 'deep' dormancy (Suszka, 1967; Grisez 1974; Finch-Savage *et al.*, 2002). Warm stratification is especially needed for underdeveloped seed prior to cold stratification (Finch-Savage, 2001; Finch-Savage *et al.*, 2002).

Wild cherry seed continues to germinate from three months to as long as two years (Grisez, 1974; Catalan, 1985; Ellis *et al.,* 1985). It grows on wide range of environments (Yaman, 2003, Eşen *et al.,* 2005). Variable seed germination behavior enhances plants survival over a wide range of habitats (Radosevich *et al.,* 1997). The 'trade-off' with seed polymorphism is less germination in 'optimal conditions' (Swanton, 2003), which might explain the generally low mean seed germination rates in the present experiment. The variable germination behavior that were observed in both artificial and natural stratification (i.e., continued germination into the second-year) in the present study might also be explained by the temperature regime to which wild cherry seeds were exposed to during stratification and germination periods (Suska, 2005, personal com.). Temperature increases during artificial or natural stratification or germination tests to 20-25^{o}C put wild cherry seeds into a secondary dormancy state. This results in a mixture of dormant and nondormant seeds, substantially decreasing cumulative germination rate at the end of germination trial (Suska, 2005, personal com.). Therefore, Suska (2005, personal com.) suggests use of a recurring temperature regime alternating between 3 and 20^{o}C for 16 and 8 hours, respectively, rather than use of a constant temperature regime for germination of wild cherry seed.

The results of the present study also showed the existence of a substantial seed-source variation within wild cherry, suggesting no broad generalizations should be made for wild cherry in Turkey, regarding level of seed dormancy, required pretreatments and germination behavior. The Ereğli seed source, the only a coastal seed source in the study, was mostly superior to the others in all pretreatments in both speed and cumulative rate of germination in spite of its relatively poor seed soundness (Table 2 and Fig. 1). Fast germination confers the seedlings of a species an advantage over its competitiors to rapidly capture resources and improves its survival rate (Radosevich *et al.*, 1997; Swanton, 2003).

The similarly great germination performances of the SS in both 120DCS and 20DWS + 15DCS +15DWS + 85DCS pretreatments might indicate that the Ereğli seeds had less ratio of underdeveloped seeds and needed a less complex pretreatment prior to germination (Table 2). Milder climatic conditions of Ereğli, as compared to those of the interior locations of other SSs might induce a lower level of seed dormancy in the seeds of SS (Grisez, 1974; Close & Wilson, 2002; Swanton, 2003). These results should however not rule out 'tree-to-tree variation' and variation within the same tree of wild cherry or seed polymorphism as significant determinants for variable germination behaviors among seed-sources (Grisez 1974; Isik, 1986, Radosevich *et al*., 1997; Finch-Savage, 2001; Finch-Savage *et al*., 2002). Thus, a cautious linkage between seed-source and germination behavior is made in the present study.

Cherries have exhibited either variable results or no substantial increase in seed germination following chemical scarification (Grisez, 1974). Only, 48-hour 0.1% citric acid exposure prior to 120-day cold stratification substantially improved black cherry seeds (Jones, 1963; Grisez, 1974). Wild cherry similarly benefited from 48-hour weak Citric acid exposure prior to a 90-day cold stratification in the present study. Exposures longer than 48 hours however decrease germination substantially, probably due to embryo damage (Grisez, 1974).

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