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Effects of different pretreatments on germination of *Prunus serotina* seed sources

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Abstract: *Establishing intensive plantations of fast growing hardwood tree species that have high market values in the forest industry can narrow the gap between Turkey's demand and the supply of quality hardwood products. Black cherry (P. serotina Ehrh.) is a fast growing hardwood species with a high market value. Introducing and intensively growing black cherry (BC) in Turkey may significantly reduce the country's quality wood shortage. Adequate seed germination constitutes the first essential step for successful establishments. In this paper, effects of different pretreatments, including artificial and natural stratification, on the seeds of different BC seed sources (SSs) were studied. Pretreatments had substantial effects on the dormancy breaking and germination behaviours of the SSs. Consecutive periods of complex warm and cold artificial stratification regimes longer than 90 days or natural stratification (where seeds were assumed to be naturally exposed to this complexity) resulted in best dormancy breaking and, in turn, germination among all pretreatments. Deeper dormancy and reduced germination rates of some BC seeds as the altitude of the source increases might suggest an ecological adaptive strategy of the species. BC may have deeper morphophysiological dormancy than is commonly believed. Seed size may have a positive effect on seed germination.*

Key words: *Black cherry, Forestry, Prunus serotina, Seed stratification, Germination*

Introduction

Establishing industrial plantations with fast-growing native and introduced hardwood species on some forest and agricultural land is recommended to narrow the gap between Turkey's demand and supply of quality hardwood (Boydak and Dirik, 1998). Black cherry (P*runus serotina* Ehrh.) is well-known for its fast growth rate and highly prized wood throughout U.S. and European markets (Grisez, 1974; Marquis, 1983; Savill, 1991; Brown *et al.*, 1996). Introducing and intensively growing BC in Turkey may significantly fulfill the country's quality wood requirement.

Successful seed germination constitutes the first essential step for successful establishment (Radosevich *et al.*, 1997). Cherries generally have deeply dormant seeds. There are great variations in the methods and lengths of pretreatments for breaking seed dormancy as well as germination behaviour, both within and among different cherry species (Suszka, 1962; Grisez, 1974; Catalan, 1985; Ellis *et al.*, 1985; Finch-Savage *et al.*, 2002). For example, wild cherry (*Prunus avium* L.) requires a very complex stratification regime with consecutive warm and cold periods (Grisez, 1974; Esen *et al.*, 2006). There is a variation in literature regarding the stratification method and period for BC seed. Generally, a period of cold stratification sufficiently breaks the dormancy of BC seed and can results in a high germination rate (86%) (Grisez, 1974). A two week warm period prior to a 189 days cold period resulted in slightly greater germination success for BC seed (90%) (Suszka, 1967). Soaking BC seeds for two days in 0.1% citric acid solution enhanced germination (89%) when compared to the mean germination rate of untreated seeds (57%) in another study (Jones, 1963).

This great variation in seed germination for BC will bring about problems with seedling propagation in forest nurseries and plantation management. Therefore, elucidating the germination behaviour and dormancy breaking of BC seed is urgently needed prior to its introduction into Turkish forestry. In addition, there has been a paucity of studies in BC seed pretreatments between the 1960s and the present. This paper evaluates data on the present germination behaviour of *Prunus serotina* seed originating from various sources in Europe and the USA in different pretreatments, including various combinations of cold and warm periods and natural stratification.

Materials and Methods

Seed material: Stones (hereafter termed seeds) of four different seed sources (SSs) of BC were obtained from Sheffield's Seed Company, Inc., NY, and Lawyer Nursery, Inc., MT, USA (Table 1). Three Virginia SSs were also supplied by the USDA Forest service northeastern forest research station. All seed lots were stored in a refrigerator at 3^{o}C for four months before the experiment.

Physical characteristics of the Seeds: The weight per 1000 seeds, seed number per kg, mean seed diameter, moisture content and seed soundness (percentage of stones with fully developed seeds) of each SS were determined. Moisture contents

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Table - 1: Geographic, harvest year and various physical seed characteristics data of different *Prunus serotina* seed sources

Seed source	Supplier	Elevation (m)	Harvest year	The weight per 1000 seeds (g)	Seed number per kg.	Mean seed diameter (mm)	Moisture of air dried seed (%)	Mean deed soundness (%)
Michigan 1, USA	Sheffield's Seed Co, Inc. USA	NA[a]	2002	83	12,121	5.2	15	100
Hundary	Lawyer nursery Inc. (USA)	NA	NA	86	11,604	5.3	11	100
Ukraine	Lawyer nursery Inc. (USA)	140-200	NA	100	10,010	5.4	11	100
Michigan 2 USA	Lawyer nursery Inc. (USA)	NA	NA	79	12,650	5.2	11	100
Virginia HE, USA [a]	Northeastern Forest Research Station (USA)	860	2003	118	8,455	5.9	10	100
Virginia ME, USA[a]	Northeastern Forest Research Station (USA)	622	2003	115	8,735	5.8	9	100
Virginia LE, USA[a]	USFS Northeastern Forest Research Station (USA)	488	2003	111	9,007	6.0	10	100

[a] HE: High elevaton ; ME: Medium elevation ; LE: Low elevation ; NA: Not available

were determined following International seed testing association rules for seed testing (ISTA, 1999). Mean seed diameter of each SS was determined on 20 randomly selected seeds. Four random samples of 100 seeds from each SS were used to determine the mean seed soundness.

Pretreatments:

Artificial sratification: Seeds were soaked in deionised water for 24 hr and then mixed with moistened peat moss in a 4:1 proportion. They were then placed in large zipped plastic bags that allow gas exchange. Four different pretreatments, including the control (no stratification), were used in this study: 90 day cold stratification (90DC), 20 day warm stratification + 90 day cold stratification 20DW+90DC), 120 day cold stratification (120DC), and 20 day warm stratification + 120 day cold stratification (20DW+120DC). These treatments aimed to compare effects of various cold periods with those of warm + cold periods. One seed lot served as the control (no pretreatment) for each SS (Table 2). Seeds were stored at 3±2^{o}C in a cold room for cold stratification, whereas a growth chamber (Nuve ID 501, Nuve, Inc., Ankara, Turkey) at 20±0.5^{o}C was used for the warm stratification. Seeds were regularly aerated and, if necessary, moistened during the pretreatments. All seeds that had undergone pretreatment 20DW+120DC germinated prematurely before the end of cold stratification. The low and medium elevation (LE and ME, respectively) SSs of Virginia also exhibited premature seed germination in the 120DC pretreatment. Hence, these particular treatments were excluded from the analysis.

Natural stratification (Fall sowing) : Seeds were first sown in rows on nursery beds and then covered by raking a 1:1:1 mix of peat moss, washed sand, and sheep manure at the Düzce Forest Nursery (40°50'N; 31°10'E; 140 m. asl), Düzce, Turkey, in early November, 2003. The SS were randomly assigned to rows. For each SS, 100 seeds were used in each of four replications. The number of total germinants for each SS was determined in late May 2004. The nursery had sandy clay loam soil with a pH of 7.2-7.5 in the rooting zone. The mean yearly precipitation was 84 cm, and the annual temperature averaged 13^{o}C (Anonymous, 1999, 2005). A seed was considered germinated when it had a 5 mm long radicle (ISTA, 1999). Cumulative germination percentage was calculated as the total number of germinants at the 28 day divided by the total number of sound seeds, multiplied by 100.

Statistical analysis:

A completely randomized design and randomized complete block design, each with four replications, were utilized for the artificial and natural stratifications, respectively. Analysis of variance (ANOVA) was used to test for the main (SS and pretreatment) as well as their interaction effects. Arcsine transformations were made on all germination data before analysis. The untransformed data were presented in the tables. The adjusted Tukey Kramer mean comparison test was utilized to separate means (Hinkelmann and Kempthorne, 1994).

Germination test:

Following each of the three dormancy breaking pretreatments and the check treatment, applied to every SS, germination tests using four replications of 100 seeds each were performed in the silviculture laboratory of Duzce Forestry Faculty in Duzce, Turkey. Seeds were placed in a moist sand medium in 18 cm glass petri dishes in a growth chamber in which the temperature was 20$\pm$0.5^{o}C for the germination test (ISTA, 1999). All the petri dishes were checked for germination on days 4, 7, 10, 21 days and 28 days and moistened as needed during the course of the test.

Results and Discussion

Cherries usually have high seed soundness, varying between 96-100% (Grisez, 1974). Similarly, all of the BC SSs had 100% seed soundness in the present study (Table 1).

The cleaned seed number per kg varies between 6,261 and 30,423, with an average of 11,839 seeds for BC (Grisez, 1974). In the present study, this parameter was in the previously reported range (Table 1). The three Virginia SSs had lower mean seed numbers per kg and greater weights per 1000 seeds than the rest of the SSs. The mean seed diameters of the Virginia SSs were also greater than those of the others. The relatively greater germination performances of the Virginia SSs, when compared to those of the others (Table 2), may suggest a positive relationship between seed size and germination for BC, as a similar correlation has been reported for other tree species (*Acacia catechu* Willd., *Acacia nilotica* Willd., Khera *et al.*, 2004).

The mean moisture contents of SSs before germination testing ranged between 9 and 15%. Seed moisture content may substantially affect germination (Grisez, 1974). The seed moisture content in the present study did not vary substantially among the SSs (Table 1) and were mostly above the critical levels (9-10%) reported by previous researchers for cherry species (Huntzinger, 1971; Grisez, 1974).

Seeds in the control did not germinate at all by the end of the experiment and were not included in statistical tests, confirming that either natural or artificial stratification is required for germination of BC seeds (Huntzinger, 1971; Grisez, 1974; Suszka *et al.*, 1996; Table 2).

At the end of the present experiment, significant SS, pretreatment, and SS x pretreatment effects were found for the cumulative seed germination percentage for the artificial pretreatments. SSs behaved significantly different in seed germination in different stratification treatments (Table 2).

The Virginia sources from low and middle elevations (VA LE and VA ME, respectively) and the Ukraine source generally had relatively high mean cumulative germination percentages. The Hungary and high elevation Virginia (VA-HE) SSs ranked intermediate. The Virginia seeds tended to germinate better as their altitudes decreased. The two Michigan sources had low seed germination (Table 2).

Although seed germination behaviour significantly varied among different SSs in the present study, a solid genetic based linkage cannot be made. First, harvest dates were not available for all of the SSs, and this might place a confounding effect on the results. The different germination behaviours of the SSs might also have been influenced by substantial tree-to-tree and even within tree variation in seed development and germination behaviour (Grisez, 1974) or seed polymorphism (Isik, 1986; Radosevich *et al.,* 1997; Swanton, 2003).

Some conclusions may yet be reached for the three Virginia SSs. As plants experience lower winter temperatures, they tend to impose deeper seed dormancy and thus need longer pretreatments before germination (Grisez, 1974; Close and Wilson, 2002; Swanton, 2003). This type of strategy in germination helps species survive to over different habitats (Radosevich *et al.*, 1997; Swanton 2003) and different years (Isik, 1986). The results, including the reduced cumulative germination rates of the Virginia seeds as their altitude increases, the increased cumulative germination, and the greatest germination speed of the VA-HE seeds in the longest (90 day cold period) pretreatment, probably indicate the adaptive behaviour of these SSs in germination under harsh environmental conditions in higher altitudes.

Among all artificial pretreatments, the complex pretreatment with consecutive warm and cold terms significantly improved mean percentage seed germination (> two-fold) for all of the SSs except for Michigan 2 (Table 2). This pretreatment, for example, brought about an almost three fold increase in the mean germination of the VA-LE SS, when compared to the 90 day long cold pre treatment. There was no significant difference in seed germination between the 90 day and 120 day long cold treatments for any seed source, except for Michigan-1 (Table 2).

BC is generally documented as a relatively straightforward cherry species in terms of seed dormancy, pretreatment requirements and germination behaviour. A four month period of moist low temperatures suffices to break seed dormancy (86%) (Grisez, 1974), and the addition of a short, warm period does not improve seed germination (90%) significantly. However, the present study suggests that the pretreatment period for BC seeds might be reduced in total by addition of a 20 day short, warm period prior to a 90 day cold period, with a significantly improved germination rate, when compared to the recommended 120 day long pretreatment (Grisez, 1974, Table 2). This result also indicates that BC may have deeper morphophsysiological dormancy than is generally believed.

SSs exhibited relatively greater germination performances in the complex artificial pretreatment when

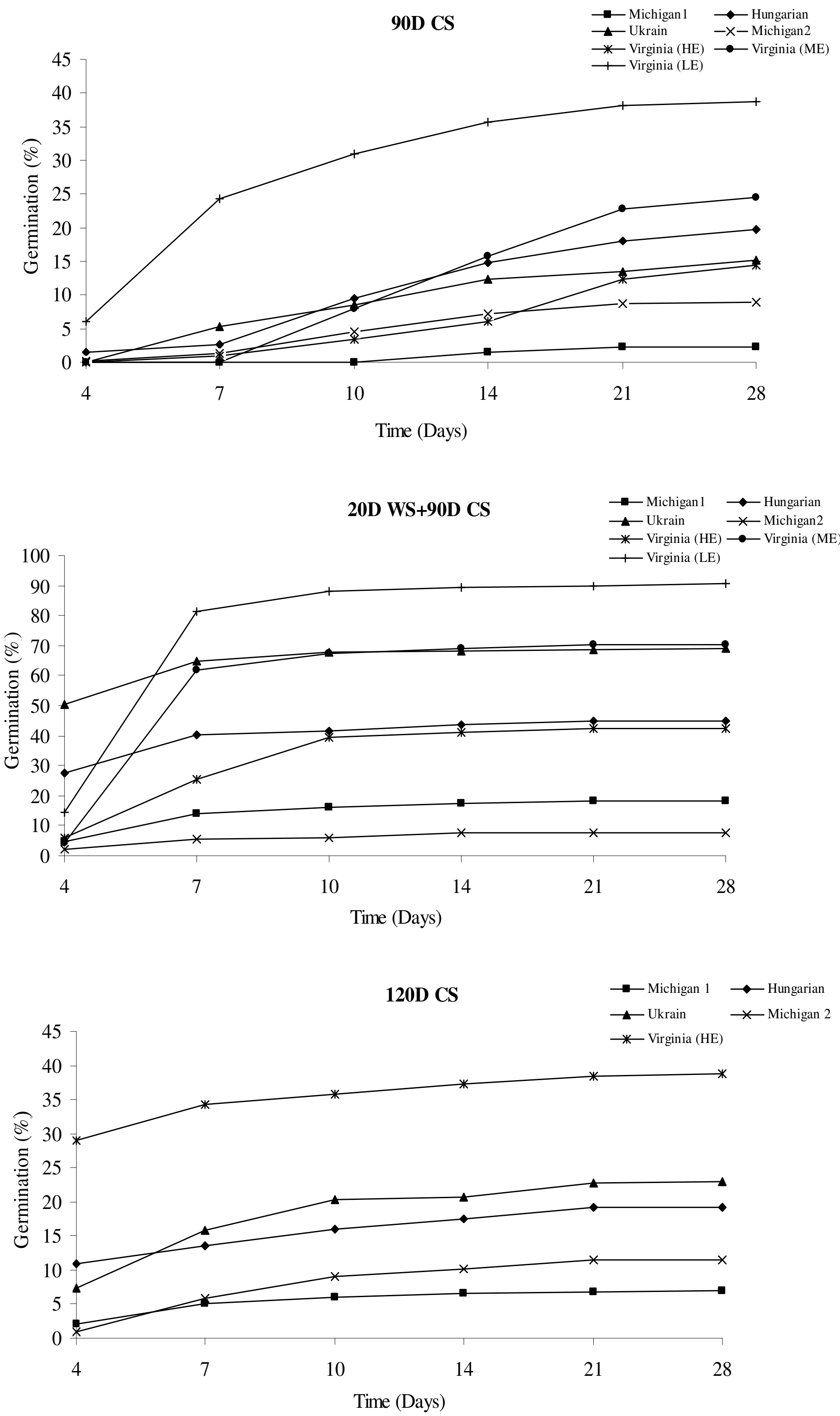

Fig. 1: Rate of germination of the seeds of different *Prunus serotina* seed sources under different

Table - 2: Effects of different artificial pretreatments on the mean cumulative germination of *Prunus serotina* seeds from different seed sources

Pretreatment[1]	Mean germination percentage (%) and standard errors (±)						
	Ukraine Europe	Hungary Europe	MI-1[2] USA	MI-2 USA	VA-HE[2] USA	VA-ME[2] USA	VA-LE[2] USA
90DC	15.3 b[3] (±3.75)	19.8 b (±3.32)	2.3 c (±0.48)	9.0 a (±0.91)	14.5 b (±2.63)	24.5 b (±3.33)	38.8 b (±13.97)
20DW + 90DC	69.3 a (±8.63)	44.8 a (±2.87)	18.3 a (±1.97)	7.8 a (±1.49)	42.3 a (±6.75)	70.3 a (±1.55)	90.5 a (±1.32)
120DC	23.0 b (±0.91)	19.3 b (±2.02)	7.0 b (±2.04)	11.5 a (±1.19)	38.8 ab (±8.41)	pg[a]	pg
No stratification (the check)	0	0	0	0	0	0	0
Grand mean[4]	**35.8**	**27.9**	**9.2**	**9.4**	**31.8**	**47.4**	**64.6**

[1] D: Days; C: Cold stratification; W: Warm stratification; pg: premature germination before the end of the stratification
[2] MI: Michigan; VA: Virginia; HE: High-elevation; ME: Intermediate elevation; LE: High-elevation
[3] Means within the same column with different letters are significantly different according to the adjusted Turkey kramer mean separation test ($p \leq 0.05$).
[4] No statistical test results were shown for this main effect due to significant seed-source x pretreatment interaction

Table - 3: Effects of natural stratification (fall sowing) on the mean cumulative germination of *Prunus serotina* seeds from different seed sources

Seed source[1]	Germination percentage (%) and standard errors (±)
Virginia ME, USA	59.5 a[2] (±9.47)
Virginia LE, USA	53.5 ab (±6.85)
Hungary	34.0 bc (±10.30)
Virginia HE, USA	30.5 bc (±6.85)
Ukraine	22.0 c (±5.23)
Michigan-2, USA	20.5 c (±4.79)
Michigan-1, USA	16.0 c (±2.94)

[1] Seed-source main effect was significant ($p \leq 0.05$)
[2] Means with different letters are significantly different ($pd \leq 0.05$).

compared to their respective performances in the natural (fall sowing) treatment, except for Michigan-2 (Table 2, 3).

Similarly to their cumulative germination rates, the SSs had variable germination speeds in different artificial stratification treatments in the present study (Fig. 1). The VA-LE seeds showed a progressively increasing germination during germination test in pretreatments 90DCS and 20DWS + 90DCS. The majority of BC seeds germinated by the end of the first week in the latter pretreatment, except for the Ukraine seeds.

The seeds from Ukraine and Hungary exhibited greater speeds of emergence than the rest of the SSs and had more than 50% of their total number of seeds germinated by day 4.

Many seeds germinated by day 4 in the 120DCS pretreatment, especially those of the VA-HE SS. Generally, germination was very low and did not culminate until the third week in the 90 day cold period treatment. The more rapid emergence rates of the Ukraine and Hungary SSs may present a competitive advantage (Radosevich *et al.*, 1997; Swanton, 2003), although the cumulative seed germination rates of these SSs ranked intermediate. Rapid emergence allows seedlings to develop quickly to usurp resources under relatively short growing periods and unfavourable growth conditions (Radosevich *et al.*, 1997; Swanton, 2003).

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