dormancy-breaking seeds of

1 **DORMANCY LEVEL AND PRETREATMENTS IN *Fraxinus ornus* subsp. *cilicica***

2 ~~SEEDS~~, an endemic to Turkey

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many comments, suggested word changes and some questions have been written on pages of the ms. These pages have been scanned and made into a pdf file.

Abstract

Fraxinus ornus subsp. *cilicica* is an endemic ornamental tree ~~grows in~~ Southern Turkey. The present study was carried out to determine the dormancy ~~level~~ and appropriate pretreatments ~~in the seeds of the species~~ and to investigate interpopulational variation in ~~terms~~ ~~of~~ seed dormancy. ~~The~~ seeds were collected from seven populations (Menzelet, Boztoprak, Düziçi, Kozan, Pozantı, Gündoğmuş, Eğirdir). The average 1000-seed ~~weight~~ was ~~calculated~~ ~~as~~ 30.5 g. Average ~~rate of~~ sound, insect-infected, and empty seeds ~~were~~ 74 %, 16.8 %, and 9.2 %, respectively. Germination percentages of populations varied significantly due mainly to different sound seed ~~rate~~. ~~The~~ study ~~showed~~ that ~~the~~ seeds of ~~the species~~ have deep physiological dormancy ~~and~~ required about 18 weeks of prechilling treatment for full dormancy removal. ~~The~~ depth of dormancy for ~~all the~~ seeds of populations was relatively similar. Warm incubation at 20 °C before the prechilling was not effective ~~on the dormancy elimination~~.

Key words: *Fraxinus ornus* subsp. *cilicica,* Taurus Flowering Ash, Seed, Dormancy, Germination

Introduction

Fraxinus ornus subsp. *cilicica* (*F.o.cilicica*), Taurus Flowering Ash, is an endemic ornamental tree scattered on Taurus Mountains in Southern Turkey. The tree mostly found on sunny southern slopes and grows from 350 m to 1500 m. Its height is usually 8-10 m ~~and~~

grows up to 20 m height (Yaltırık 1978; Browicz 1984). Research on the seed physiology of this species is needed before widespread seedling production and ex-situ conservation of *F.o.cilicica* can be successfully carried out.

Fraxinus ornus (Oleaceae), also known Manna ash (Fraxigen 2005), is an insect-pollinated tree (Verdú et al. 2006). *F.o.cilicica* fruits are elongated, winged, single-seeded samaras that are borne in clusters (Bonner 2002). *F. ornus* has male and hermaphrodite trees in a breeding population and only hermaphrodites produce fruits (Dommée et al. 1999; Verdú 2004; Verdú et al. 2007).

Seed dormancy is a very prevalent characteristic of species in temperate regions of the world. Level of dormancy varies greatly depending on species, population location, and individuals within the populations (Bewley and Black 1994; Baskin and Baskin 1998). Depth of dormancy in the seeds of *Fraxinus* species varies greatly and need two to seven months cold stratification at 4±2 °C for dormancy removal depending on the species (ISTA, 1996; Suszka et al. 1996; Bonner 2008).

The main objectives of this exploratory study were to determine dormancy level and appropriate pretreatments in endemic *F.o.cilicica* seeds from seven populations throughout its distribution and to investigate interpopulational variation of seed dormancy of the species.

Materials and Methods

The samaras were collected from seven populations in Turkey (Table 1). In the laboratory, firstly, samaras were dried to about 8 % moisture content (MC). MC of seeds was determined by low temperature oven method, 17 h at 104±1 °C (ISTA, 1996). MC was expressed as the percentage of the fresh weight of the seed. Three replicates of 150 (3x50) seeds were cut lengthwise to find out the sound, insect-infected, and empty seed rate. For each

population, 1000-seed weights ~~have been~~ calculated from 800 (8x100) seeds according to ISTA (1996) rules.

Table 1. Seed origins of *F.o.cilicica* used in the study.

Populations	Latitude	Longtitude	Altitude (m)
Menzelet (K.Maras)	37°41'	36°50'	700
Boztoprak (K.Maras)	37°32'	36°18'	950
Düziçi (Osmaniye)	37°16'	36°30'	1400
Kozan (Adana)	37°31'	35°52'	380
Pozantı (Adana)	37°22'	34°53'	1150
Gündoğmuş (Antalya)	36°49'	32°00'	950
Eğirdir (Isparta)	37°44'	30°50'	1450

***Dormancy Level and Pretreatment Requirements*:** To find out the dormancy level and prechilling requirement of the species, seeds from seven different populations (Table 1) were subjected to six different pretreatments (Table 2). Pretreatments were applied in the plastic bottles covered with perforated aluminum folio under dark conditions. The top of the bottle was covered with perforated aliminum foil for gas exchange. Both warm incubation and prechilling were applied without media. Seeds were hydrated to maximum MC (maximum MC of *F.o.cilicica* seeds is about 50 %) by daily water spraying for ~~three~~ days and then dried back to 40-42 % MC. During the preatments, the bottles were weighed weekly to check for altered moisture content of seeds and distilled water was added by spraying if needed. MC of seeds during the pretreatments without media should generally be about 8-10 point below the maximum MC of the seeds (Yilmaz 2006). Warm incubation was applied at 20 °C.

Table 2. The pretreatments applied in the experiment.

4-w warm incubation + 10-w prechilling
4-w warm incubation + 14-w prechilling
4-w warm incubation + 18-w prechilling
10-w prechilling
14-w prechilling
18-w prechilling

Germination Test : ~~The~~ germination tests were performed on two ~~layered~~ filter paper in 15-cm diameter petri dishes ~~with 150 (3*50) seeds~~ at 5/15 °C ~~alternating temperatures~~

which is the appropriate germination temperature for the prechilled seeds of *F.o.clicica* (Yilmaz and Tonguç, in press). Seeds were rinsed with distilled sterile water for ~~five~~ 5 minutes prior to the germination test to remove dust from the seed surface. The seeds were considered to be germinated when their radicles protruded 3 mm and showed geotropism. The petri dishes were examined every ~~two~~ 2 days, ~~and the~~ germinated seeds were counted and removed. Germination tests were terminated on day 28.

Germination Parameters : In the germination tests, germination percentage (GP) and mean germination time (MGT) were calculated according to the following formulas (Bewley and Black, 1994):

$$GP(\%) = \frac{\sum n_i}{N} x100$$

Where GP(%) is the germination percentage, n_i is the number of germinated seeds at week i, and N is the total number of incubated seeds per test.

$$MGT = \frac{\sum (t_i . n_i)}{\sum n_i}$$

Where MGT is the mean germination time, t_i is the number of weeks from the beginning of the test, and n_i is the number of germinated seeds recorded on week $t_{(i)}$

Statistical Analyses:

GP and MGT values of treatments were subjected to factorial ANOVA to detect the significance of population, germination temperature and stratification duration. Percentages of sound, insect-infected, and empty seed ~~were~~ also ~~taken~~ to factorial ANOVA to check ~~the~~ for provenancial differences. Percent values (sound, insect-infected, and empty seed rate, GP) ~~was~~ were transformed using arcsine square root ($\sqrt{P}$) to normalize error distribution prior to

variance analyses. When a significant effect was detected, differences among the groups were identified using Duncan's New Multiple Range test. There were no germination after "4-w warm incubation + 10-w prechilling" and "10 w prechilling" pretreatments and the results of both pretreatments were not included in the statistical analyses.

Results

1000-seed weights of *F.o.cilicica* varied from 23.6 gr to 36.2 gr. Average 1000-seed weight of seven populations was 30.5 gr.

The soundness of the seeds from different populations varied significantly (Table 3). The average percentage of sound, insect-infected, and empty seeds were 74.0 %, 16.8 %, and 9.2 %, respectively.

Table 3. 1000-seed weights and the percentages of sound, insect-infected, and empty seeds from different populations.

Populations	1000-seed weight* (g)	Sound (%)	Insect infected (%)	Empty (%)
Menzelet	35.5	89.3 a	6.0 a	4.7 b
Boztoprak	31.9	94.0 a	4.7 a	1.3 a
Düziçi	36.2	81.3 b	8.7 a	10.0 b
Kozan	27.0	70.7 c	24.0 b	5.3 b
Pozantı	32.6	72.7 bc	22.0 b	5.3 b
Gündoğmuş	27.0	73.3 bc	18.0 b	8.7 b
Eğirdir	23.6	36.7 d	34.0 c	29.3 c
Average	30.5	74.0	16.8	9.2

* Air dry weight, about 8 % moisture content.
[1]no statistical differences between the same letter in the same column ($p < 0.05$)

Both pretreatment and population significantly affected the germination percentage of *F.o.cilicica* seeds. The pretreatment x population interaction effect was found to be significant on GP (Table 4).

Table 4. Effects of pretreatments and population on germination percentage (GP) and mean germination time (MGT) of *F. ornus* subsp. *cilicica* seeds, results of factorial ANOVA.

Source	df	GP			MGT		
		MS	F	P-value	MS	F	P-value
Pretreatment	3	2729.1	338.7	0.000	107.26	281.21	0.000

There was very distinct difference between GP of "14 w prechilling" and "18 w prechilling" treatments (Figure 1). The part of ungerminated seeds after the "4 w warm incubation + 14 w prechilling" and "14 w prechilling" pretreatments were found to be sound while the ungerminated seeds after the "4 w warm incubation + 18 w prechilling", and "18 w prechilling" pretreatments were either decayed or infected.

There was distinctive difference between the average GP of populations. Menzelet and Boztoprak populations demonstrated the highest average GP while the overall GP of Eğirdir population was 14.8 %, evidently lower than those of other populations due to a lower germination potential (Figure 2).

need to add another column to this table showing germination of Fresh (control) non treated seeds

Table 5. Germination percentages of *F.o.cilicica* seeds after different pretreatments.

Populations	Germination percentage (%)				Average
	4 w warm incubation + 14 w prechilling	14 w prechilling	4 w warm incubation + 18 w prechilling	18 w prechilling	
Menzelet	56,7 b[1]	47,3 b	87,3 a	90,0 a	70,3 A[3]
Boztoprak	50,7 c	45,3 c	77,3 b	90,7 a	66,0 A
Düziçi	54,7 b	19,3 c	85,3 a	79,3 a	59,7 B
Kozan	34,7 b	36,7 b	65,3 a	62,7 a	49,8 C
Pozantı	24,7 d	41,3 c	62,7 b	74,0 a	50,7 C
Gündoğmuş	46,0 c	62,0 b	59,3 b	78,7 a	61,5 B
Eğirdir	6,0 c	7,3 c	20,7 a	25,3 a	14,8 D
Average	39,1 C[2]	37,0 C	65,4 B	71,5 A	

[1] The values on the same line followed by the same letters are not significantly different at $p < 0.05$.
[2] The values on the same line followed by the same capital letters are not significantly different at $p < 0.01$.
[3] The values on the same column followed by the same capital letters are not significantly different at $p < 0.01$.

were the nongerminated seeds still dormant or dead?

The period of prechilling also affected the germination speed. The longer the prechilling period, the faster the germination occurred. While the average MGT was 16.4 day after 14-w prechilling, ~~MGT value~~ it was 12.4 day after 18-w of prechilling (Table 6). The 4-w warm incubation before the prechilling treatment didn't affect the average germination speed. Population factor wasn't generally effective on the MGT at different pretreatments (Table 4).

Population	6	1776.2	220.5	0.000	10.21	26.76	0.000
Pretreatment * Population	18	121.9	15.1	0.000	1.18	3.11	0.001
Error	56	8.1			0.38		

Neither the nor the

~~Both~~ "4 w warm incubation + 10 w prechilling" ~~and~~ "10 w prechilling" pretreatments resulted in any ~~did not produce~~ germination.

significant

There were ~~apparent~~ differences between prechilling treatments (Table 5; Figure 1). ~~The~~ and the highest germination percentages were ~~14-w prechilling treatment was insufficient for complete escape from dormancy while 18-w~~ obtained for seeds receiving 18 weeks of ~~prechilling treatment totally eliminated seed dormancy and GP found to be the highest at this~~ prechilling. ~~treatment.~~

Average GPs were 39.1 %, 37.0 %, 65.4 %, and 71.5 % at "4 w warm incubation + 14 w prechilling", "14 w prechilling", "4 w warm incubation + 18 w prechilling", and "18 w prechilling", respectively. For the germination of the seeds, at least 14-w prechilling treatment was necessary. delete – can see this in table

is this needed?

A 4-w warm incubation prior to 14 w and 18-w prechilling treatments didn't affect the overall average GP (Table 5). However, Duziçi population demonstrated ~~the~~ a higher germination percentage at "4-w warm incubation + 14-w prechilling" than "14-w prechilling" treatment. The 4-w warm incubation prior to prechilling decreased the germination rate of Pozantı and Gündoğmuş populations which have more insect infected seeds and as a result significant amount of seeds from these populations either decayed or were dead after 4-w incubation duration.

did not

? or percentage?

you need to express germination percentages and rates based on sound seeds. or add a column to table 5 showing % viable seeds,

The average GP after the "4 w warm incubation + 18 w prechilling", and "18 w prechilling" treatments were 65.4 % and 71.5 % and did not differ significantly. The GP of Menzelet and Boztoprak populations reached to 90.0 % and 90.7 % while the maximum GP of Eğirdir population was 25.3 % at 18 w prechilling treatment.

what was this?

Only Eğirdir population with the lowest germination potential germinated slower than other populations.

Table 6. Mean germination times of *F.o.cilicica* seeds after different pretreatments.

Population	Mean Germination Time (day)				Average
	4 w warm incubation + 14 w prechilling	14 w prechilling	4 w warm incubation + 18 w prechilling	18 w prechilling	
Menzelet	15,2 b[1]	15,5 b	12,0 a	12,1 a	13,7 A[3]
Boztoprak	16,0 c	16,2 c	11,5 b	12,8 a	14,1 AB
Düziçi	15,9 c	17,6 b	11,7 a	12,2 a	14,3 B
Kozan	16,3 b	16,6 b	12,3 a	12,7 a	14,5 B
Pozantı	16,1 c	16,0 c	12,9 b	11,4 a	14,1 AB
Gündoğmuş	16,4 c	15,0 b	12,2 a	11,8 a	13,8 AB
Eğirdir	18,6 b	18,1 b	14,5 a	14,1 a	16,3 C
Average	16,3 B[2]	16,4 B	12,4 A	12,4 A	

[1] The values on the same line followed by the same letters are not significantly different at $p < 0.01$.
[2] The values on the same line followed by the same capital letters are not significantly different at $p < 0.01$.
[3] The values on the same column followed by the same capital letters are not significantly different at $p < 0.01$.

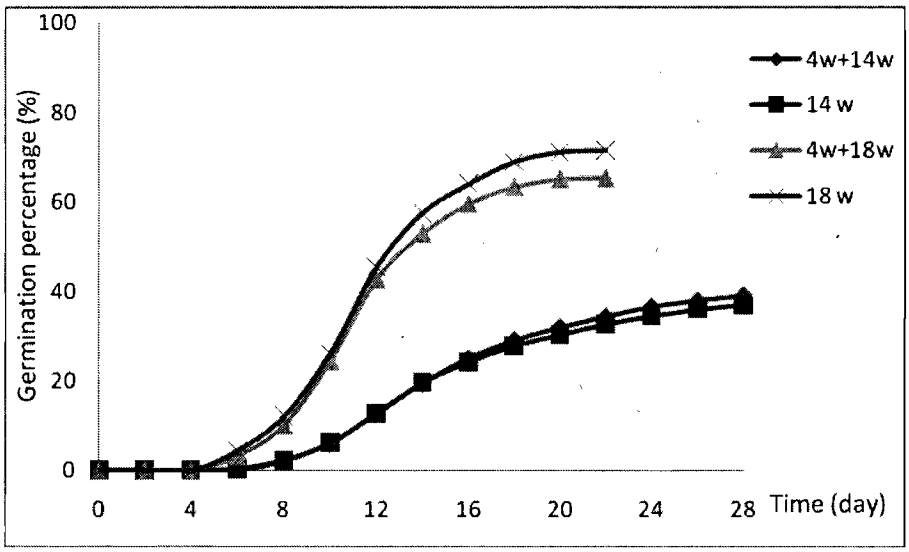

Figure 1. Average germination percentages of *F.o.cilicica* seeds from seven populations after four different pretreatments, @ 5/15 °C.

in Light or dark?

at

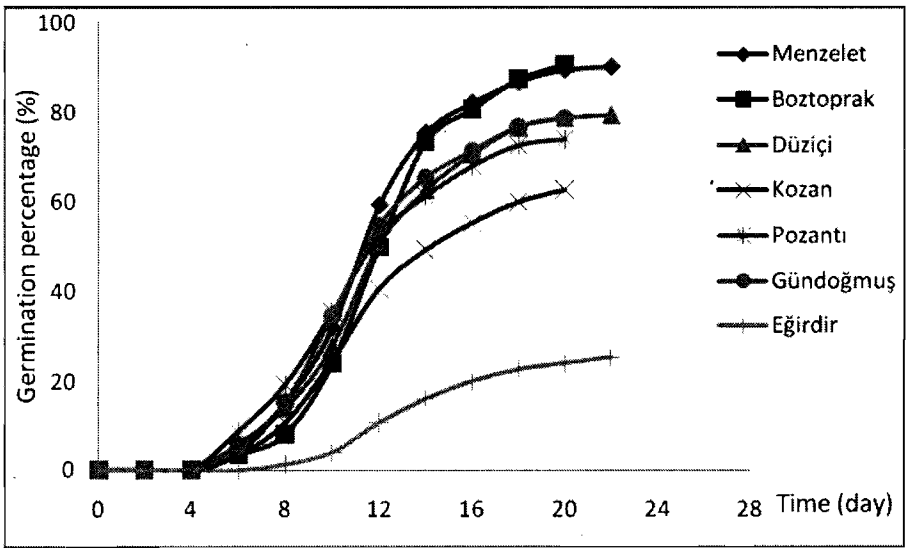

Figure 2. Germination percentages of seven populations after 18-w prechilling, @ 5/15 °C.

Discussion

This study ~~demonstrated~~ that the seeds of *F.o.cilicica* have deep physiological dormancy, About 18-w prechilling ~~was~~ needed for full elimination of the seed dormancy ~~before the germination. 4 w~~ warm incubation at 20 °C ~~was~~ not effective ~~on the~~ dormancy ~~elimination.~~

Depth of dormancy generally varies according to species, species locations, or individuals in the same site (Bewley and Black 1994; Copeland and McDonald 1999). Prechilling durations up to 14-w weeks was clearly insufficient for the complete elimination of dormancy while the seeds exhibited their full germination potential after 18-w prechilling. After the full elimination of dormancy with 18-w prechilling, seeds collected from the highest (Eğirdir, 1450 m) and the lowest altitudes (Kozan, 380m) demonstrated relatively lower germination. The seeds from Eğirdir clearly had the lowest germination percentage than seeds from the other populations after all the pretreatments, likely because of a low germination ability arising from its highest altitudinal and furthest westerly and northerly location. Insect

infection was main reason in relatively lower germination percentages of Pozanti (74.0 %) and Gündoğmuş (78.7 %) populations. Need to tell reader what % of sound seeds germinated,

The general ~~pretreatment~~ dormancy-breaking requirement of *F. ornus* seeds is 2 to 8 weeks warm incubation and 8 to 15 weeks prechilling (Piotto 1994; Piotto and Di Noi 2003). In this study, the warm incubation was not effective on *F.o.cilicica* seeds (Table 5). Further, 10-w and 14-w prechilling ~~was~~ were clearly insufficient in ~~the~~ complete ly eliminating dormancy while 18-w prechilling treatment totally eliminated the dormancy.

Seeds of some ash species (*F. excelsior, F. americana, F. nigra, F. pennsylvanica*) have morphophysiological dormancy (Villiers and Wareing 1965; Suszka et al. 1996; Bonner 2008), and warm incubation + prechilling ~~are usually applied in elimination~~ can break some levels of morphophysiological dormancy (Baskin and Baskin 2004). However, warm incubation is not recommended for *F. ornus* (Draghici and Abrudan 2011). Similarly, ~~four~~ 4 weeks incubation at 20 ° C before the 10, 14 and 18-w prechilling treatments did not significantly affect ~~the~~ germination. As a result of this experiment, ~~it could be concluded~~ we suggest that *F.o.cilicica* seeds have deep physiological dormancy ~~but don't~~ and do not have ~~considerable~~ morphological dormancy. Did you look at the embryo in fresh seeds? Is it large or small?

The cold-wet-strafication method in dormancy elimination of seeds is applied in a material such as sand or peat at 3± 2 ° C. In this method, the pretreatment is usually stopped when the seeds start to germinate and the pretreatment durations become insufficient for some part of the seeds due to the heterogenity of dormancy level of seed lot (Suszka et al. 1996). In ~~this~~ our? study, both warm incubation and prechilling treatments are applied without medium. When the level of MC of some seeds during the prechilling is controlled at 8-10 point lower than maximum level, the prechilling duration could be extended without any germination until the elimination of dormancy from all the seeds (Muller et al. 1999 and Yilmaz 2006). ~~This~~ Our? study demonstrated that the level of moisture content can be effectively controlled at 40-

42 %, 8-10 point lower than maximum level, for the prechilling treatments in *F.o.cilicica* seeds. The prechilling without medium has been also successfully used in *F. excelsior* seeds (Tylkowski 1990; Suszka et al. 1996).

Seed dormancy is closely related to the natural distribution of plant species (Schmidt 2000; Alvarez-Aquino and Williams-Linera 2002; Fenner and Thompson 2005). In a study on *F. angustifolia* seeds, it was found that seeds originated from southern Italy had lower level of dormancy than the northerly populations (Piotto and Piccioni 1998). In ~~this~~ study, the depth of dormancy was relatively similar for all the populations (Table 5). There were significant differences among the populations in terms of GPs due mainly to different sound seed~~rate~~. At the end of the 18-w prechilling, Egirdir (25.3%) and Kozan (62.7%) populations had exhibited the lower GPs than the other populations.

The embryos of *F. ornus* (Arrillaga et al. 1992), *F. excelsior* and *F. angustifolia* (Raquin et al. 2002) germinate at high rates at in vitro conditions without applying prechilling ~~p~~treatments. The effects of pericarp, seed coat, and endosperm on dormancy should be separately investigated on *F.o.cilicica* seeds.

Acknowledgment

~~This study~~ supported by The Scientific and Technological Research Council of Turkey, Project Number: 107 O 624.

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