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Effects of Stratification, Temperature and Storage on Germination in Three Provenances of *Fraxinus angustifolia* subsp. *oxycarpa* Seeds

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Abstract: The effects of stratification, temperature and storage on seed germination performance were investigated in 3 provenances of *Fraxinus angustifolia* subsp. *oxycarpa* seeds. In the first study, seeds were subjected to 9 different periods of cold stratification or warm stratification followed by cold stratification to overcome dormancy. Stratification and provenances affected seed germination percentage (GP) and germination value (GV) when seeds were germinated in darkness at 30/20 °C. Sixteen weeks' cold stratification (GP = 40.0, GV = 3.8), 4 weeks' warm stratification + 4 weeks' cold stratification (GP = 43.7, GV = 4.2) and 4 weeks' warm stratification + 8 weeks' cold stratification (GP = 45.8, GV = 4.3) were the most efficient in promoting germination performance, as well as dormancy removal. In the second study, the effects of storage at +4 °C and -5 °C for 12 months on the quality of pretreated seeds were investigated. Seeds from each provenance stored at +4 °C were germinated during storage, while seeds stored at –5 °C had high germination parameters after storage. In the third study, dormant seeds stored at –5 °C and +4 °C for 12 months were subjected to stratification (4 weeks of warm + 4 weeks of cold stratification) and then germinated at 30/20 °C, 20 °C and 25/4 °C. Germination performance was not reduced by storage temperature in any provenances but was significantly affected by germination temperature, and the 25/4 °C regime always gave the highest germination parameters (GP = 60.8, GV = 6.6).

Key Words: *Fraxinus angustifolia* subsp. *oxycarpa*, seed dormancy, pretreatment, seed storage

Katlama, Sıcaklık ve Saklamanın Üç Farklı Kaynaktan Elde Edilmiş *Fraxinus angustifolia* subsp. *oxycarpa* Tohumlarının Çimlenmesi Üzerine Etkisi

Özet: Türkiye'de üç ana yayılış alanından temin edilen *Fraxinus angustifolia* subsp. *oxycarpa* tohumlarının çimlenme potansiyelleri üzerine katlama işlemi, saklama ve sıcaklığın etkisi incelenmiştir. Araştırmanın birinci safhasında; tohumların bir bölümü soğuk katlama ve sıcak katlama + soğuk katlama şeklinde 9 farklı ön işleme tabi tutulmuştur. Bu işlemleri takiben 30/20 °C'de yapılan çimlendirme denemelerinde, çimlenme yüzdesi (GP) ve çimlenme değerinin (GV) orijin ve işlem tarafından önemli oranda etkilendiği belirlenmiştir. Üç orijinin ortalaması alındığında en yüksek çimlenme parametreleri; 16 hafta soğuk katlama (GP = 40.0, GV = 3.8), 4 hafta sıcak + 4 hafta soğuk katlama (GP = 43.7, GV = 4.2) ve 4 hafta sıcak + 8 hafta soğuk katlama (GP = 45.8, GV = 4.3) işlemleri sonucunda elde edilmiştir. Araştırmanın ikinci safhasında; çimlenme engeli 4 hafta sıcak + 4 hafta soğuk katlama sonucu giderilen tohumlar +4 °C ve –5 °C'de 12 ay süre ile saklanmıştır. Bu işlem sonucu; +4 °C'de saklanan tohumlarda saklama süresince ön çimlenme görülmüştür. –5 °C de saklanan tohumların ise saklamayı takiben çimlenme değerlerinde az bir düşüş belirlenmekle birlikte, çimlenme yüzdelerinde istatistiksel anlamda bir düşüş tespit edilmemiştir. Araştırmanın üçüncü safhasında; ön işleme tabi tutulmayan tohumlar –5 °C ve +4 °C'de 12 ay saklandıktan sonra 4 hafta sıcak + 4 hafta soğuk katlama işlemini takiben üç farklı sıcaklıkta (30/20 °C, 20 °C ve 25/4 °C) çimlendirilmiştir. Çimlendirme sonucunda; saklamanın her üç orijinde de çimlenme performansını etkilemediği fakat çimlendirme sıcaklığının önemli etkisinin olduğu tespit edilmiştir. En yüksek çimlenme parametreleri 25/4 °C'de yapılan çimlendirme denemeleri sonucu (GP = 60.8, GV = 6.6) elde edilmiştir.

Anahtar Sözcükler: *Fraxinus angustifolia* subsp. *oxycarpa*, tohum çimlenme engeli, ön işlem, tohum saklama

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Introduction

Fraxinus angustifolia Vahl. has 3 subspecies in Turkey and *F. angustifolia* subsp. *oxycarpa*, which is a fast growing tree and has valuable wood (Çiçek, 2002), is the most common taxon (Anşin and Özkan, 1993). Seed dormancy, whose effect may be released by moist pretreatments, was observed in several forest species (Bewley and Black, 1994) and mature seeds of most *Fraxinus* spp. (ash) are frequently deeply dormant. Seeds of ash often have pericarps impermeable to oxygen, immature embryos, metabolic inhibitors in the endosperm and embryos, and deficiencies of growth-promoting substances in embryos (Villiers and Wareing, 1964; Bonner, 1974; Tinus, 1982; Nowag, 1998). Dormant seeds can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements. As a result of variable causes of dormancy among ash species, recommendations for treating ash seeds to break dormancy or stimulate germination are quite variable and include stratification, after-ripening followed by stratification, water soaking, treatment with a variety of growth regulatory chemicals, and others (Villiers and Wareing, 1964; McBride and Dickson, 1972; Bonner, 1974; Walle, 1987; Nowag, 1998; Piotto and Piccini, 1998). In *Fraxinus* spp., the stratification treatment consisting of a period of warm moist stratification at 15-20 ºC prior to an identical period of cold moist stratification at 3-5 ºC is considered by many authors to be more effective (Bonner, 1974; Tylkowski, 1990; Piotto, 1994; Nowag, 1998). In nature the beneficial effect of cold stratification is obtained during the winter, and in the nursery the same result may be achieved by keeping seeds at low temperatures, generally within a range from 1 to 5 °C, mixed with moist peat or sand (Bradbeer, 1988; Bonner et al., 1994). Seeds generally have an inherent high genetic variability, which results in great heterogeneity in their behavior and, in particular, in their germinability following stratification procedures. The degree of dormancy may be expected to show some variation related to climate of origin, and seed dormancy can vary considerably between different clones and from seed lot to seed lot (Edwards, 1980). The International Seed Testing Association (ISTA) (1993) prescribes a single pretreatment to overcome dormancy in all ash tree seeds: 2 months of warm stratification at 20 ºC followed by 7 months of cold stratification at 3-5 ºC. However, this treatment would appear too long taking into consideration the usual practices followed in nurseries.

The fundamental micro-environmental factor for achieving high germination is the temperature at which the seeds resume growth, whether pretreated or not. In some species the alternating temperature itself stimulates germination (Gordon and Edwards, 1991). The ISTA (1993) rules prescribe alternating temperatures of 8 h at 30 ºC / 16 h at 20 ºC, in darkness, during germination tests in *Fraxinus* spp. However, evidence exists regarding a better response of seeds of *Fraxinus* spp. to a wider daily temperature range, i.e. alternating 20/3 ºC or 25/5 ºC (Suszka, 1978; Piotto, 1994; Piotto and Piccini, 1998).

Following collection, for some hardwood species, pretreated seeds are dried to 8-12% moisture content and stored in sealed containers at temperatures varying from 0 ºC to –5 ºC for a few years (Suszka, 1975; Grzeskowiak and Suszka, 1983; Muller and Bonnet-Masimbert, 1989a; Tylkowski, 1990). However, the storage and viability of pretreated (non-dormant) *F. angustifolia* subsp. *oxycarpa* seeds have not been studied intensively.

It is important to understand the effect of stratification, temperature and storage on germination both for practical nursery applications and for conservation, but the methods to stimulate germination for *F. angustifolia* subsp. *oxycarpa* have not been fully studied yet. Thus, the objectives of the current study were to determine a) germination response of *F. angustifolia* subsp. *oxycarpa* seeds from 3 provenances to 9 different stratification treatments, b) effects of storage on the germination of dormant and pretreated seeds, and c) effects of temperature on seed germination.

Materials and Methods

Seed source

Seeds from Demirköy (41º 49' N, 27º 56' E, 20 m above sea level), Hendek (40º 52' N, 30º 36' E, 25 m above sea level) and Sinop (42º 02' N, 35º 05' E, 20 m above sea level) were collected in October, 2002. All provenances were packed in plastic bags and transported to the laboratory. Initial viability was obtained using the ISTA (1993) tetrazolium method. Seeds were randomly sampled for all experiments.

Stratification

For stratification treatments damaged or unusually small seeds were discarded and the remaining seeds were soaked for 24 h in room conditions (about 20 ºC) in running water, and afterwards mixed with moistened sand. They were then subjected to varying periods of stratification (Table 1). Based on the results of stratification, the seeds stored at 4 ºC until use were stratified (4 weeks of warm stratification at 20 ºC followed by 4 weeks of cold stratification at 4 ºC) and sown in early April, 2003 at the Düzce Forest Nursery, Turkey (140 m altitude, 40^{o} 50' N, 31^{o}10' E), and the percentages of seedlings were recorded. Nursery soil was sandy-clay loam with soil pH of 7.15-7.52 (E.T.L.M., 1999).

Storage of pretreated seeds

In order to overcome dormancy, seeds were subjected to 4 weeks of warm stratification followed by 4 weeks of cold stratification. After stratification treatment, chosen because of its success in dormancy-breaking in a previous comparison of 9 pretreatments, seeds were separated from the sand and exposed to a gentle air stream at room temperature (at about 20 °C) until their moisture content was reduced to 11 ± 1%, expressed as percentages of fresh weights. Seed moisture content was determined by oven drying 4 subsamples of 5 g each at 103 ± 2 °C for 17 ± 1 h (ISTA, 1993). Pretreated seeds were then placed in hermetically sealed bottles and stored at –5 and +4 °C. To determine the changes in seed germination performance, the germination test was carried out in darkness at 30/20 °C after 12 months of storage.

Storage of dormant seeds and effects of temperature on the germination

Freshly harvested seeds were dried at room temperature to 11 ± 1% moisture content before the storage of seeds. For the storage of dormant seeds damaged or unusually small seeds were discarded, and the remaining dried seeds were stored in sealed bottles at –5 ºC and +4 °C for 12 months. After soaking in water for 24 h, the seeds stored for 12 months were subjected to 4 weeks of warm stratification at 20 °C followed by 4 weeks of cold stratification at 4 °C. Viability of the stratified seeds was evaluated in the dark under 3 temperature regimes of constant 20 ºC and alternating 25/4 ºC or 30/20 ºC, as prescribed in the ISTA (1993) rules.

Germination test

Germination tests using 4 replications of 50 seeds each were performed in darkness at a temperature regime of 8 h at 30 °C plus 16 h at 20 °C (30/20), as prescribed in the ISTA (1993) rules in all experiments except for temperature treatments. Germinations were counted every day until the 56th day and seeds were considered germinated when they exhibited 5 mm long radicles (Walle, 1987; ISTA, 1993). Germination percentage (GP) was calculated for each day and for the 56th day. Mean germination time (MGT), a speed index, was computed as follows: $MGT = \Sigma(n_i x d_i)/\Sigma n$ where n is the total number of germinated seeds during the 56-day germination test, n_i is the number of germinated seeds on day d_i and d_i is the number of days during the germination period (between 0 and 56 days) (Younsheng

Table 1. Pretreatments to overcome dormancy in *Fraxinus angustifolia* seeds.

Pretreatments[1]			
1.	Control	:	no stratification
2.	4C	:	4 weeks of cold stratification
3.	8C	:	8 weeks of cold stratification
4.	12C	:	12 weeks of cold stratification
5.	16C	:	16 weeks of cold stratification
6.	2W + 4C	:	2 weeks of warm + 4 weeks of cold stratification
7.	4W + 4C	:	4 weeks of warm + 4 weeks of cold stratification
8.	4W + 8C	:	4 weeks of warm + 8 weeks of cold stratification
9.	8W + 28C	:	8 weeks of warm + 28 weeks of cold stratification

[1]Pretreatments: C = cold stratification at 4 ºC; W = warm stratification at 20 ºC.

and Sziklai, 1985). Quicker germination corresponds to lower values of MGT. Germination value (GV), which reflects both speed and completeness of germination, was determined for each treatment according to the equation given by Djavanshir and Pourbeik (1976), GV = (ΣDGS/N) x GP x 10, where DGS is daily germination rate calculated as the cumulative number of seeds germinated by daily intervals divided by the number of days since the beginning of the test, N is the number of DGS calculated during the test, and GP is the final germination percentage of the seeds.

Statistical analysis

Final germination percentage, germination value and mean daily germination were calculated for each trial. The significance of treatment means was tested by factorial analysis on the transformed arcsin square root of germination percentages using SPSS for Windows (Zar, 1996). Germination values and mean germination times were also subjected to ANOVA. Means were tested by Duncan's multiple range test when significant differences were identified. A significance level of 5% was used for all statistical analysis and the results obtained from experiments were analyzed separately.

Results and Discussion

Effects of stratification on dormancy breakage

The tetrazolium tests showed that the initial seed viability among populations varied between 65% (Hendek) and 74% (Demirköy). According to this result, the viability of seeds was high, indicating that they would be capable of germination under favorable conditions and that failure to germinate would be attributable mainly to seed dormancy. The ANOVA results showed that GP and GV were significantly affected by provenances and stratification treatments ($P < 0.05$). The interaction between provenances and stratification was not significant. Germination response of seeds kept under stratification for different time durations is shown in Table 2. A low germination was observed with a general mean of 19.5% in non-stratified seeds after being maintained at 30/20 ºC in darkness for 56 days and the lowest germination was observed in the Hendek provenance (13% germination). Pretreatments greatly influenced GP within provenances as well as the general average ($P < 0.05$). The highest GP was obtained from pretreatments 5 (16C), 7 (4W + 4C) and 8 (4W + 8C) when averaged over the 3 provenances. In Hendek

Table 2. Germination percentage (GP) and germination value (GV) under a 30/20 °C regime for 3 provenances of *F. angustifolia* subsp. *oxycarpa* seeds.

Pretreatments	Demirköy		Sinop		Hendek		Average	
	GP	GV	GP	GV	GP	GV	GP	GV
1. Control	19.5a[1]	0.4a	25.0a	0.6a	13.0a	0.1a	19.0a	0.4a
2. 4C	28.0b	0.9a	29.5ab	1.1a	16.0a	0.6a	24.5b	0.9a
3. 8C	25.5b	1.3b	32.0b	1.6b	23.5b	1.4b	27.8b	1.4b
4. 12C	39.5c	2.8c	35.5b	2.2c	29.0c	2.0bc	34.0c	2.3c
5. 16C	45.0cd	4.4e	43.0c	4.2e	31.0c	2.7c	40.0cd	3.8d
6. 2W + 4C	40.0c	3.4d	35.5b	3.2d	33.5c	2.9c	36.7c	3.2cd
7. 4W + 4C	47.5d	4.5e	44.5c	4.3e	39.0d	3.8d	43.7d	4.2d
8. 4W + 8C	51.5d	4.8e	43.5c	4.2e	42.5d	4.0d	45.8d	4.3d
9. 8W + 28C	pg		pg		pg			

Pretreatments: C = cold stratification; W = warm stratification (numbers refer to length of stratification in weeks).

pg: pregermination before the end of the stratification.

[1]Means in the same column followed by the same letter are not significantly different at $P < 0.05$.

provenance pretreatment 5 was not as effective as pretreatments 7 and 8, but was as effective as pretreatments 7 and 8 in Demirkoy and Sinop provenances (Table 2).

GVs also differed among the various stratification treatments (Table 2). Due to a significant reduction in germination rates under different stratification duration, pretreatments 5 (16C), 7 (4W + 4C) and 8 (4W + 8C) resulted in maximum GV and improved germination far better than the other treatments within provenances as well as the general average.

In *F. excelsior*, the pretreatment considered by many authors to be most effective consists of 8-18 weeks' warm stratification (15-20 °C) followed by an identical period of cold stratification (12-16 weeks) at 3-5 °C (Tylkowski, 1990; Nowag 1998); in *F. ornus* successful results have been achieved with cold stratification of 12-16 weeks, following 2-4 weeks of warm stratification (Bonner, 1974; Suszka, 1978; Piotto, 1994). Although seeds of *F. angustifolia* are generally considered to have a shallow degree of dormancy, 1-4 months' cold stratification or 4 weeks' warm stratification prior to 4-8 weeks' cold pretreatments usually remove dormancy and induce rapid germination depending on seed provenances (Piotto, 1994; Piotto and Piccini, 1998). In the present study, 4 weeks' warm stratification followed by 4 weeks' cold stratification (pretreatment 7) was the quickest way to promote speed and completeness of germination and this can potentially reduce seed dormancy and enhance germination in the 3 provenances. The ISTA (1993) prescribes a single pretreatment to suppress dormancy in all *Fraxinus* tree seeds: 8 weeks of warm stratification (20 °C) followed by 28 weeks of cold stratification (3-5 °C). This treatment to remove dormancy in *Fraxinus* seeds appears too long, and in the present study pretreatment 9 (8 weeks of warm stratification followed by 28 weeks of cold stratification), recommended by the ISTA (1993) was shown to be ineffective for *F. angustifolia* subsp. *oxycarpa* because of pregermination during the stratification periods in all provenances, as determined in some ash species by Piotto and Piccini (1998).

Comparison among provenances across all pretreatments indicated variability, and Demirkoy was significantly superior to the others. This variability in germination ability among different sources is generally attributed to either the genetic characters of the source population (Bewley and Black, 1994) or the impact of the mother plant environment (Fenner, 1991; Andersson and Milberg, 1998).

Seeds subjected to 4 weeks of warm stratification followed by 4 weeks of cold stratification were sown in the nursery, and seedling percentages of Demirköy, Sinop and Hendek provenances after 2 months were 54%, 50% and 42%, respectively, and seedlings grown in the nursery appeared normal in the fall.

Effects of pretreated seed storage on germination performance

When the stratified seeds were stored at 4 °C, pregermination occurred during storage, which is why these treatments were excluded from the test.

Germination percentage of pretreated seeds (4 weeks' warm stratification followed by 4 weeks' cold stratification) was not significantly affected by 12 months of seed storage at –5 °C, but the GV and germination rate of all provenances were significantly reduced after storage when the stored seeds were germinated at 30/20 °C (Table 3). Comparison of GPs, GVs and germination rates (averages for the 3 provenances) of pretreated seeds stored for 12 months at –5 °C showed no significant differences among GPs but a better performance by the controls (0 months' storage) for GV and germination rate. The stored seeds showed slower germination in comparison with the controls in all provenances. The better behavior of the control seeds, particularly in terms of rate (MGT), could be due to the fact that they were not subjected to drying after breaking dormancy while seeds stored for up to 12 months could have suffered some damage from the storage process.

For some species, to avoid undesirable germination during storage, seeds are pretreated to allow breaking of dormancy but avoid germination, and the seeds are then dried to an 8-12% moisture content and stored in sealed containers at temperatures varying from –3 to –5 °C. Such a method allows storage of pretreated seeds of *Fagus sylvatica* for 5 years (Suszka, 1975; Muller and Bonnet-Masimbert, 1989b) and for 2-4 years in the case of *F. excelsior* and *Prinus avium* (Suszka, 1978; Grzeskowiak and Suszka, 1983; Muller and Bonnet-Masimbert, 1989a; Tylkowski, 1990). The effects of drying and storage on the quality of *F. angustifolia* pretreated seeds subjected to 2 weeks of warm stratification + 2 weeks of cold stratification were

Table 3. Germination percentage, germination value and mean germination time 3 provenances of *F. angustifolia* subsp. *oxycarpa*, under a 30/20 °C temperature regime for pretreated seeds stored at –5 °C for 12 months.

Provenances	Length of storage (months)					
	Germination percentage		Germination value		Mean germination time	
	0	12	0	12	0	12
Demirköy	47.5a	42.5a	4.5a	3.8b	30.6a	34.3b
Sinop	44.5a	41.0a	4.3a	3.7b	31.5a	33.7b
Hendek	39.0a	35.0a	3.8a	2.6b	30.3a	34.7b
Average	43.7a	39.5a	4.2a	3.4b	30.8a	34.2b

Means in the same row followed by the same letter are not significantly different at $P < 0.05$.

investigated by Piotto (1997). After 4, 8 and 12 months of storage at –3 °C, significant differences were observed in the germination rate.

The present study showed that pretreated seeds of *F. angustifolia* subsp. *oxycarpa* cannot be stored at +4 °C for 12 months but can be dried to 11 ± 1% moisture content and stored at –5 °C for at least 12 months without serious quality losses.

Effects of dormant seed storage and temperature regime on germination performance

One-year dormant stored seeds were stratified (4 weeks' warm stratification followed by 4 weeks' cold stratification) and germinated under 3 temperature regimes. Comparison of GPs and GVs within provenances at 30/20 °C under 0-month storage and 12-month storage at –5 °C and +4 °C showed that storage duration and storage temperature did not significantly affect germination parameters (Table 4), and the GPs and GVs of the seeds remained high after 1 year of seed storage at both storage temperatures.

The effect of the germination temperature regime was also evaluated in 1-year stored seeds at –5 °C and +4 °C, separately for each provenance, through GPs and GVs (Table 4). These parameters were significantly influenced by temperature regime for each provenance ($P < 0.05$). The provenances tested in the present study responded similarly to the various temperature regimes and a temperature regime of 25/4 °C always gave the highest final GP and GV in each provenance, followed by a 30/20 °C temperature regime.

When germination data were combined over all provenances and storage temperatures after 12 months of storage, the GPs and GVs were significantly affected by the temperature regimes of 25/4 °C, 30/20 °C and 20 °C (Table 5). The 25/4 °C temperature regime produced about 60% germination and a 6.6 GV averaged over the 3 provenances and 2 storage temperatures, which is better germination than the germination temperature of 30/20 °C prescribed by the ISTA (1993). These results indicate that a temperature regime of 25/4 °C seems to be more beneficial to germination of the seeds. The prescribed 30/20 °C and 20 °C are less than optimal for the germination of *F. angustifolia* subsp. *oxycarpa* seeds. A temperature regime of 20/3 °C or 25/5 °C was also found to be more beneficial to germination for some *Fraxinus* species, and suggested a probable need for modification of the ISTA rules for testing *Fraxinus* species (Suszka, 1978; Piotto, 1994; Piotto and Piccini, 1998).

Conclusions

From our results, seeds of *F. angustifolia* subsp. *oxycarpa* are confirmed to be in a dormant state. The results obtained from pretreatments 5 (16 weeks' cold stratification), 7 (4 weeks' warm stratification followed by 4 weeks' cold stratification) and 8 (4 weeks' warm stratification followed by 8 weeks' cold stratification) are statistically homogeneous and the most efficient at promoting speed and completeness of germination, as well as dormancy removal (Table 2). When translated into practical application of the present results, the

Table 4. Germination percentage and germination value of *F. angustifolia* subsp. *oxycarpa* seeds, under 3 temperature regimes, stored at -5 and +4 °C for 12 months.

Provenances	Germination temperature (ºC)	Storage duration (months)					
		Germination percentage			Germination value		
		0	12		0	12	
			Storage temp. (°C)			Storage temp. (°C)	
			-5	+4		-5	+4
Demirköy	20		21.5c[1]	18.5c		0.5c	0.4c
	25/4		66.5a	63.5a		7.2a	6.8a
	30/20	47.5A[2]	49.5bA	46.5bA	4.5A	4.7bA	4.4bA
Sinop	20		14.0c	19.0c		0.2c	0.3c
	25/4		61.5a	62.5a		6.7a	6.8a
	30/20	44.5A	41.0bA	45.5bA	4.3A	4.0bA	4.2bA
Hendek	20		18.5c	15.0c		0.3c	0.1c
	25/4		57.0a	56.0a		6.3a	6.2a
	30/20	39.0A	37.0bA	38.5bA	3.8A	3.9bA	3.9bA

[1]Means followed by the same letter in the same column, within each provenance, are not significantly different at $P < 0.05$.
[2]Means followed by the same capital letter in the same row are not significantly different at $P < 0.05$.

Table 5. Germination percentage and germination value (averages for 3 provenances and 2 storage temperatures) of *F. angustifolia* subsp. *oxycarpa* seeds after 12 months of storage.

Germination temperature (ºC)	Germination percentage	Germination value
20	17.7a	0.3a
25/4	60.8c	6.6c
30/20	43.0b	4.2b

Means in the column followed by the same letter are not significantly different at $P < 0.05$.

nurseryman can consistently shorten pretreatment length, e.g., from 16 weeks' cold stratification or from 12 weeks (4 weeks' warm + 8 weeks' cold stratification) to 8 weeks (4 weeks' warm + 4 weeks' cold stratification) when facilities for warm stratification are available. Seedlings in the nursery raised from stratified seeds (4 weeks' warm stratification followed by 4 weeks' cold stratification) appear normal.

Storage of pretreated (non-dormant) seeds at +4 ºC for 12 months reduced germination performance and pregermination occurred during storage, but storage at –5 ºC did not reduce GPs significantly despite causing a slight decrease in GV and germination rate (Table 3). This technique can represent a useful tool in nursery management since planning sowing is much easier when seeds are ready to sow and because surplus stratified seeds can be easily stored for future use.

Storage of dormant seeds at –5 and +4 ºC for 12 months did not reduce germination performance combined over all provenances. When seeds were germinated at 25/4 ºC, 20 ºC and 30/20 ºC, the 25/4 ºC temperature regime always gave the highest germination performance. Germination temperature of 30/20 ºC, prescribed by the ISTA (1993), was found to be suboptimal in this research. Knowledge of optimal germination temperature not only proves helpful when germination takes place in controlled environments (growth chambers, greenhouses, etc.), but also permits better scheduling of stratification treatments, so that their completion coincides with the best sowing periods (Piotto and Piccini, 1998). Stratified seeds (4 weeks' warm stratification followed by 4 weeks' cold stratification) of *F. angustifolia* subsp. *oxycarpa* should be sown in nurseries during periods of the year characterized by fairly wide fluctuations in temperature, e.g., a 25 °C day and a 4 °C night during early spring (late March-early April), to obtain the best germination results.

Further research is needed to investigate the feasibility of storage of untreated seeds in polyethylene bags or other nonpermeable containers for longer durations and to determine the optimum drying procedure or seed moisture content (before and during storage) in order to maximize the germination performance of pretreated seeds.

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