AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Hort Agrobot Cluj, 2011, 39(1):283-287

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

# The Effect of Different Stratification Conditions on the Germination of *Fraxinus angustifolia* Vahl. and *F. ornus* L. Seeds

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**Abstract**

The aim of the paper was to assess the impact of different stratification treatments recommended by the literature on the shortening of manna ash (*F. ornus* L.) and narrow-leaved ash (*F. angustifolia* Vahl.) seed germination in order to identify a suitable pre-germination treatment for seedling production. Seeds of three Southern Romanian origins for both species were exposed to four stratification treatments (cold (3°C) and warm (20°C), with and without sand-peat medium). The highest percentage of germination (up to 87% for *Fraxinus ornus* and up to 71% for *F. angustifolia*) was recorded in the case of the stratified seeds with cold medium (3°C) and relatively constant seed moisture (between 45% and 65%) throughout the treatment.

***Keywords:*** cold treatment, dormancy, *Fraxinus ornus, Fraxinus angustifolia*, seed

## Introduction

Among the broadleaves found in Romanian lowland forests, the species belonging to *Fraxinus* genus are remarkable both for their timber value and for their silvicultural role (Haralamb, 1967). The most common native European *Fraxinus* species (*F. excelsior* L., *F. angustifolia* Vahl. and *F. ornus* L.) grow naturally in the country and cover more than 60,000 ha together with two rarer species (*F. pallisae* Wilmot. and *F. coriariifolia* Scheele) (Fraxigen, 2005; Şofletea and Curtu, 2007). In the present socio-economic and forest ownership context of Romania, these species are important both for conservation and forest development purposes (Ioras and Abrudan, 2006; Şofletea *et al*. 2007; Şofletea *et al*. 2007; Abrudan *et al*., 2009, Ioras *et al*., 2009; Stancioiu *et al*., 2010).

Seeds have an inherent high genetic variability, which determine a great heterogeneity in their behavior and in their germination. Seed dormancy was observed in several species of *Fraxinus* genus (Bewley and Black, 1994), the mature seeds of which are frequently deeply dormant. *Fraxinus* spp. seeds often have pericarps impermeable to oxygen, metabolic inhibitors in the endosperm and embryos, immature embryos or deficiencies of growth-promoting substances in embryos (Villiers and Wareing, 1965; Bonner, 1974; Tinus, 1982; Nowag, 1998). The degree of dormancy shows some variation related to the climate of origin and seed lot (Edwards, 1980).

Dormant seeds can be stimulated to germinate using treatments that satisfy certain physiological requirements. The recommendations for treating *Fraxinus* spp. seeds to break dormancy or stimulate germination are quite diverse and include stratification, after-ripening followed by stratification, water soaking, the treatment with a variety of growth regulatory chemical etc. (Villiers and Wareing, 1964; Marshall, 1981; Walle, 1987; Piotto and Piccini, 1998). In *Fraxinus* spp., the stratification treatment consisting of a period of warm moist stratification at 15-20°C prior to a period of cold moist stratification at 3-5°C is considered by many authors to be effective (Bonner, 1974; Tylkowski, 1990; Piotto, 1994; Nowag, 1998). The International Seed Testing Association (ISTA) prescribes a single pretreatment to overcome dormancy in all *Fraxinus* ssp. seeds: 2 months of warm stratification at 20°C followed by 7 months of cold stratification at 3-5°C (ISTA, 1993). However, this treatment would appear too long taking into consideration the usual practices followed in nurseries.

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 ranging from 1 to 5° C, mixed with moist peat or sand (Bradbeer, 1988).

The main environmental factor for achieving high germination is the temperature at which the seeds resume growth, whether pretreated or not; in some species an alternating temperature stimulates germination (Gordon and Edwards, 1991). ISTA rules (ISTA, 1993) prescribe alternating temperatures of 8 h at 30° C / 16 h at 20° C, in darkness, during the germination tests in *Fraxinus* spp. However, a better response of *Fraxinus* spp. seeds of to a wider daily temperature range (i.e. alternating 20/3° C or 25/5° C) was reported by several authors (Suszka, 1978; Piotto, 1994; Piotto and Piccini, 1998).

For nursery practice purposes it is important to identify an effective stratification method that allows for seed sowing next spring after harvesting. In this paper we assessed the impact of four different stratification conditions on the germination of *Fraxinus ornus* and *Fraxinus angustifolia* seeds, in order to identify a suitable pre-germination treatment for seedling production.

**Materials and methods**

Seeds of both species were collected in the period 26-29 October 2009 from three origins in Southern Romania (Tab. 1).

A tetrazolium test was performed in order to assess the initial viability of seeds from each origin, according to ISTA provisions (4 repetitions of 100 seeds) (ISTA, 1993; Draghici and Abrudan, 2011), after the manual grading of seeds and the removal of the sterile ones at the beginning of November 2009.

In order to establish a suitable moisture for germination, seed imbibition was evaluated by gravimetric method at room temperature (Parascan *et al*., 1975), using for each origin of the two species three repetitions of 50 seeds, as described by Draghici and Abrudan (2011).

The seeds were kept for 10 weeks after harvesting in the refrigerator at a relatively constant temperature of 4 °C; afterwards four stratification conditions/treatments were applied for each species and origin:

• **Treatment 1 (T1): Cold treatment (3°C) with medium.** Four repetitions of 100 seeds were mixed with a sand/peat medium in a volume ratio of 1:3 and were placed in plastic containers. The medium consisted of an equal proportion of sand and peat, having a relative humidity of 29% (wetting degree U6=wet) (Târziu, 1997), which was measured with the ECH2O EC-5 sensors for soil moisture.

After weighing, the containers were kept at a temperature of 3°C in the seed conservation centre of ICAS Braşov (Draghici and Abrudan, 2011). Medium moisture was assessed daily, by weighing and filling with water the deficit that occurred from the previous day. Once a week the seeds were removed from containers and medium aeration and homogenization were performed. At the same time, the seed moisture was measured with a thermo balance (MB 45).

• **Treatment 2 (T2): Warm treatment (20°C) with medium.** The seeds (4x100) of each origin were kept at 20° C, in the sand/peat medium, placed in plastic containers. The assessment/monitoring procedure was the same as in the case of the cold treatment.

• **Treatments 3 and 4 (T3 and T4): Cold (3°C) and warm (20°C) treatments without medium.** Two groups of seeds (4x100 each) were kept in parallel in two climate rooms. In the first climate room the temperature was set at 20°C and the relative humidity of air at 95% and in the second climate room a temperature of 3°C was maintained at an 80% relative humidity of air. The maximum humidity allowed by the climate room was chosen in both cases because in previous tests it was observed that seeds lost a large amount of water when placed in the climate room.

After stratification, the germination tests were performed in darkness at a temperature of 3°C. The germination percentage was calculated for each species and origin whilst the potential differences between origins were assessed by one-way ANOVA.

**Results and discussions**

The initial seed viability varied between 69.75% and 88,25% for *F. angustifolia* and between 81.5% and 90% for *F. ornus* (Tab. 2). These relatively high values indicated that seeds would be capable of germination under favorable conditions and that failure to germinate would be attributable mainly to seed dormancy.

The results of the imbibition test resulted in similar water absorption curves (Fig. 1) for each origin of the two species; in the first days, a fast water absorption was recorded, followed by a period of low absorption. Based on these results, all seeds from treatments 3 and 4 (without medium, in the climate rooms) were soaked for 48 hours in water in order to reach a moisture of around 50% before being placed in the climate rooms.

Keeping the seeds in containers with sand/peat medium at high temperature (20°C) resulted in their decay after 15 weeks of treatment, so this method proved to be ineffective.

Tab. 1. The origin of manna ash (*F. ornus* L.) and narrow-leaved ash (*F. angustifolia* Vahl. ) seeds

| Seed Origin | Forestry Directorate | Forest District | Management Unit | Sub-compartment | Altitude (m) |
|---|---|---|---|---|---|
| *F. ornus* L. | | | | | |
| Răcari | Târgovişte | Răcari | IV Răcari | 28C | 136 |
| Drăgăşani | Vâlcea | Drăgăşani | III Dobruşa | 80B | 205 |
| Balş | Slatina | Balş | V Bistriţa | 137B | 145 |
| *F. angustifolia* Vahl. | | | | | |
| Sadova 1 | Craiova | Sadova | III Lunca Jiului | 36B | 45 |
| Sadova 2 | Craiova | Sadova | III Lunca Jiului | 73A | 44 |
| Răcari | Târgovişte | Răcari | IV Racari | 2A | 137 |

Tab. 2. The initial seed viability

| Species | *F. angustifolia* Vahl. | | | *F. ornus* L. | | |
|---|---|---|---|---|---|---|
| Seed Origin | Sadova 1 | Sadova 2 | Răcari | Răcari | Drăgăşani | Balş |
| Seed viability (%) | 88.25 | 87.50 | 69.75 | 90.00 | 83.25 | 81.50 |
| The 1000-seed weight (g) | 90.11 | 82.35 | 100.46 | 24.73 | 31.48 | 27.90 |

Unfavourable results (no germination) were also recorded when applying the two treatments without sand/peat medium (in climate rooms at 3°C / 80% air humidity and 20°C / 95% air humidity respectively). In both treatments, seed humidity decreased very sharply (Fig. 2), possibly due to the air ventilation in the climate rooms.

Seed moisture remained relatively constant throughout the cold treatment with medium, ranging between 56% and 64% for *F. angustifolia* and between 45% and 53% for *F. ornus*.

The cold treatment with medium was the only one which provided satisfactory seed germination (Fig. 3).

The treatment period was relatively shorter for *F. ornus* and the first seed sprouted in the same period (after 14 weeks of cold treatment) for all three origins, despite the fact that significant differences ($p<0.05$) in germination percentage were recorded between Răcari (87%) and Drăgăşani (69%) origins. *F. angustifolia* seeds started to germinate after 22 weeks and for unknown reasons only the seeds of one origin (i.e. Răcari) germinated (71%).

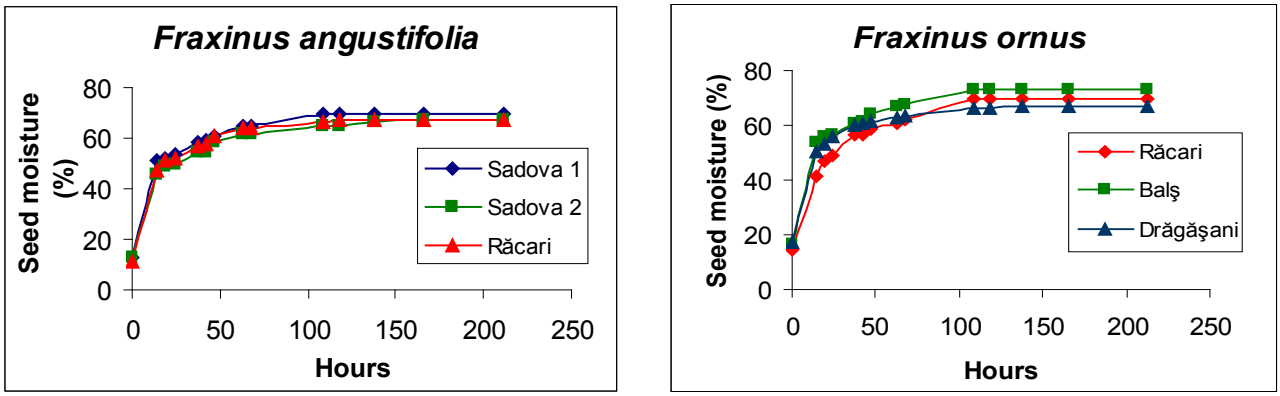
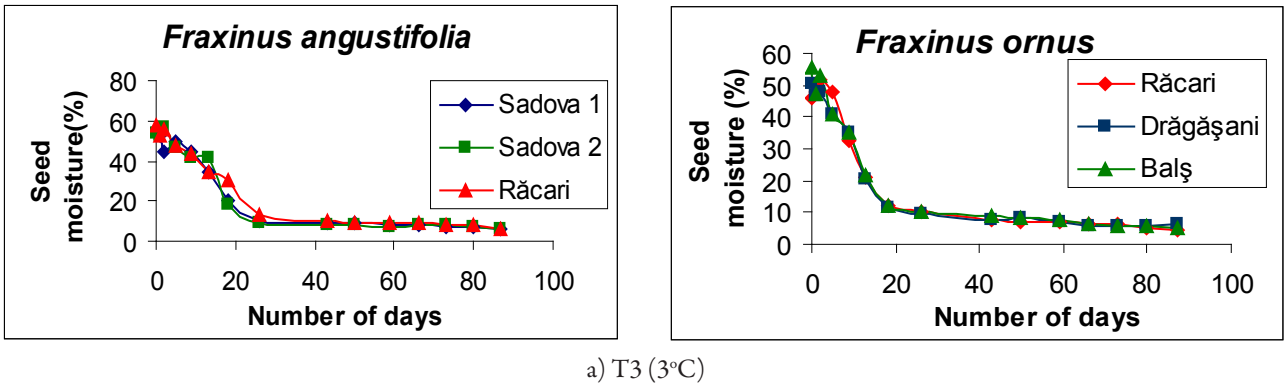
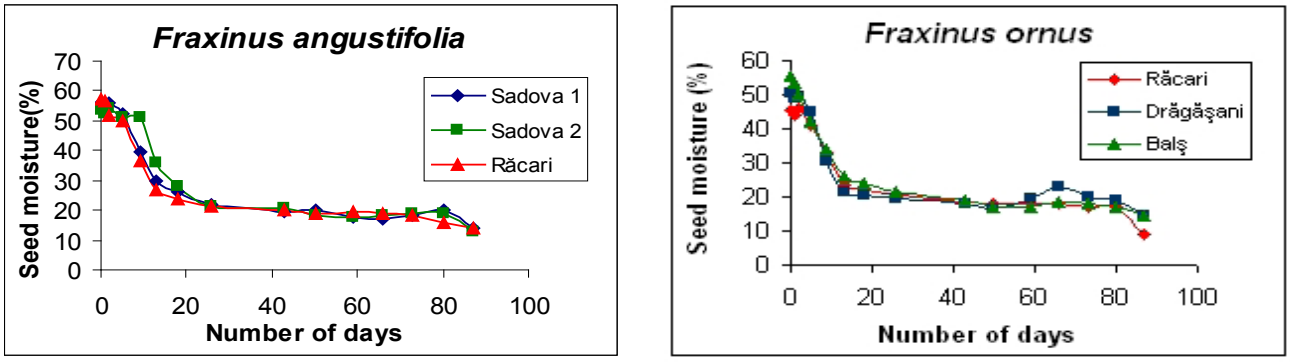



Fig. 1. Water absorbtion by seeds




a) T3 (3°C)




b) T4 (20°C)

Fig. 2. The evolution of seed moisture in the treatments without medium (a-T3 and b-T4) in climate room

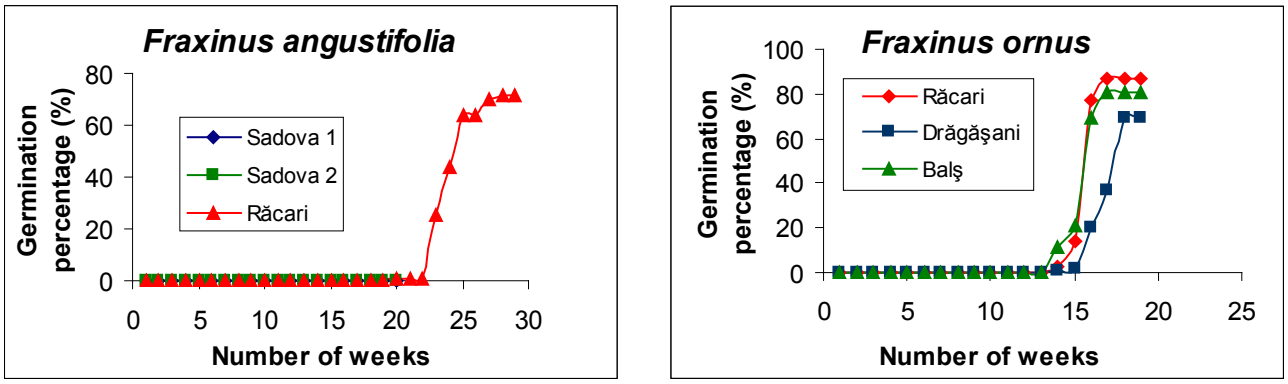



Fig. 3. Evolution of seed germination in the cold treatment (3°C) with medium

## Conclusions

Keeping the seeds of *F. ornus* and *F. angustifolia* in a sand/peat medium, at low temperature (3°C) and a relative constant humidity level (between 45% and 65%) could be an efficient and relatively simple treatment to overcome seed dormancy. Under such conditions, the seeds of *F. ornus* could start to germinate up to 7 weeks earlier than those of *F. angustifolia*.

Warm treatment is not recommended for breaking seed dormancy in *Fraxinus* spp. as the seeds decay after 3-4 months of stratification.

*Acknowledgments*

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed by the European Social Fund and by the Romanian Government under the contract number POSDRU/6/1.5/S/6.

## References

Abrudan IV, Marinescu V, Ionescu O, Ioras F, Horodnic SA, Sestras R (2009) Developments in the Romanian forestry and its linkages with other sectors. Not Bot Hort Agrobot Cluj 37:14-21.

Bewley JD, Black M (1994). Seeds: Physiology of Development and Germination. Plenum Press, New York.

Bonner FT (1974). *Fraxinus*. In: Seeds of Woody Plants in the United States. USDA Forest Service, Agricultural Handbook 450, Washington DC.

Bradbeer WJ (1988). Seed Dormancy and Germination. Blackie Acad., Glasgow.

Draghici C, Abrudan IV (2011). Preliminary results on breaking seed dormancy of manna ash (*Fraxinus ornus* L.). In: Forest and Sustainable Development. Transilvania University of Brasov Ed (in press).

Edwards DGW (1980). Maturity and quality of tree seeds - a state of the art review. Seed Sci Technol 8:625-657.

Fraxigen (2005). Ash species in Europe: Biological characteristics and practical guidelines for sustainable use. A Summary of Findings from the Fraxigen Project EU Project EVKCT00108. Oxford Forestry Institute, University of Oxford, UK.

Gordon AG, Edwards DGW (1991). Testing the germination of tree and shrub seeds, p. 5-8. In: Tree and Shrub Seed Handbook Gordon AG, *et al.* (Eds.). The International Seed Testing Association, Zurich.

Haralamb AT (1967). The culture of the forest species. Agro-Silvică Ed., Bucharest (in Romanian).

Ioras F, Abrudan IV (2006). Romanian forestry sector-privatisation facts. The International Forestry Review 8(3):361-367.

Ioras F, Abrudan IV, Dautbasic M, Avdibegovic M, Gurean D, Ratnasingam J (2009). Conservation gains through HCVF assessments in Bosnia-Herzegovina and Romania. Biodiversity and Conservation 18:3395-3406.

ISTA (1993). International rules for seed testing 1993. Seed Sci Technol. 21:160-186.

Marshall PE (1981). Methods for Stimulating Green Ash Seed Germination. Tree Planters' Notes, p. 9-11.

Nowag A (1998). Management of seed dormancy in *Fagus sylvatica*, *Fraxinus excelsior* and *Prunus avium*. Comb Proc Intern Plant Propag Soc 48:192-198.

Parascan D, Danciu M, Lungescu E (1975). Botany and Plant Physiology. Practical works. University from Brasov Ed. (in Romanian).

Piotto B (1994). Effects of temperature on germination of stratified seeds of three ash species. Seed Sci Technol 22:519-529.

Piotto B, Piccini C (1998). Influence of pretreatment and temperature on the germination of *Fraxinus angustifolia* seeds. Seed Sci Technol 26(3):799-812.

Stancioiu PT, Abrudan IV, Dutca I (2010). The Natura 2000 ecological network and forests in Romania: implications on management and administration. The International Forestry Review 12(1):106-113.

Suszka B (1978). How to achieve a simultaneous germination of afterripened hardwood seed? Proceedings of the Symposium on Establishment and Treatment of High Quality Hardwood Forests in the Temperate Climatic Region.

Nancy-Champenoux, INRA, France, p. 30-40.

Şofletea N, Curtu AL (2007). Dendrology. Dendrology. Transilvania University of Brasov Ed (in Romanian).

Şofletea N, Spârchez G, Târziu D (2007). Bioaccumulation potential of common ash (*Fraxinus excelsior* L.) depending on the ecological characteristics of the specific forest sites representative for its native distributionl range. Journal of Forests (Revista Padurilor) 1:3-9 (in Romanian).

Şofletea N, Târziu G, Spârchez D (2007). Assessment of phenotypic qualitative and quantitative traits of common ash (Fraxinus excelsior L.) depending on site and stand conditions. Journal of Forests (Revista Padurilor) 5:3-10 (in Romanian).

Tarziu D (1997). Soils and Forest Sites. Ceres Ed., Bucharest(in Romanian).

Tinus RW (1982). Effects of dewinging, soaking, stratification and growth regulators on germination of green ash seed. Can J For Res 12:931-935.

Tylkowski RW (1990). Mediumless stratification and dry storage of after-ripened seeds of *Fraxinus excelsior* L. Arbor Kornic 35:143-152.

Villiers TA, Wareing PF (1965). Dormancy in fruits of *Fraxinus excelsior*. J Exp Bot 15:359-367.

Walle C (1987). Germination uniformity of *Fraxinus excelsior* controlled by seed water content during cold treatment. Physiol Plant 69:645-650.