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Mediumless stratification and dry storage of after-ripened seeds of *Fraxinus excelsior* L.

Abstract

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The process of dormancy breaking in the seeds of European ash can take place during stratification without medium when the moisture content of samaras is maintained within the range 52.5 - 60.0% and when the same temperature regime is provided as during stratification of seeds in a medium (20°/3°C, 16+16 weeks). When after naked stratification (at moisture level 52.5% and 55.0%) the samaras are partially dried (to ~9%), they maintain their full readiness to germinate even after 80 weeks of storage at −3°C. The results reported here should however be confirmed by studies on broader seed material.

Additional key words: germination, seed moisture content.

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INTRODUCTION

In nursery practice the spring sowing of woody plants characterized by deep dormancy has to be preceded by stratification to overcome the dormant state. The traditional stratification treatment depends on placing seeds in a moist environment, at a temperature somewhat above 0°C and with the access of air. When having a large number of stratified seeds the work associated with the proper dormancy breaking is cumbersome in view of the large mass of the stratification medium (sand, peat etc.) that is needed and which has to be regularily mixed with the seeds several times to aerate and water losses have to be replenished. In western Europe, where the winter is more mild than in Poland, the stratification is conducted in concrete boxes under open air. The seeds placed with not too much sand are subjected to the atmospheric conditions (rain, low temperature) which provide more or less satisfactory stratification conditions (Bärtels 1982). In the case when seeds germinate too early or when the soil in the nursery is not ready yet, the seeds are placed in coldrooms below 0°C until sowing time.

Studies undertaken earlier in Kórnik (Suszka 1975, Suszka and Zięta 1976) and in France (Bonnet-Masimbert and Muller 1983) on dormancy breaking

in beech nuts and in seeds of maple and sycamore (Tylkowski 1989) in mediumless stratification conditions with a controlled level of moisture content as well as studies on the storage of ash seeds in a partially dried state already after dormancy breaking (Tylkowski 1988) are being continued. In the present paper results are presented of experiments conducted in this field on seeds of European ash (*Fraxinus excelsior* L.).

MATERIALS AND METHODS

Samaras of ash have been collected on October 31st 1986 in Poznań from 4 trees growing near highway with a relatively high level of traffic. After collection the samaras were partially dried at room temperature to 9.3% of moisture content and stored 11 weeks in sealed bottles at −3°C.

After storage the samaras were placed in plastic boxes and moistened gradually with tap water to four levels of moisture content (m.c.): 52.5%, 55.0%, 57.5% and 60.0% mixing them well in the boxes. Note was taken to make sure water never stay at the bottom of the boxes. Initially the watering was conducted 3 times daily until the required moisture was obtained. It was attained only after several days in view of the slow process of water imbibition by the swelling seeds. The moistened seeds/samaras in the boxes were covered with a perforated lid and placed in a phytotron chamber at a temperature of 20°C for 16 weeks and then at 3°C for the next 16 weeks. At the 20°C temperature m.c. of the samaras was controlled daily, replenishing the water losses to the previously determined weight of box with the seeds (on the basis of their dry weight and the wanted moisture level). At 3°C this was done once a week.

After 4 weeks at 20°C on the surface of the samaras molds began to appear, thus from that time onwards the water losses were replenished with a 1% water solution of the fungicide Benlate, and at the same time decayed seeds were removed. Benlate was used till the symptoms of molding persisted, after which again only water was used to replenish the boxes. At 3°C there was no need to use the fungicide.

After stratification without medium a part of the samaras from each of the moisture levels has been subjected to a germination test in a moist sand-peat medium at a cyclic temperature of 3°~25°C (16+8 h) in four replicates with 50 seeds each, and the remaining samaras were partially dried to three different m.c. levels (Table 1). The partial drying of the most moist samaras (having 60% m.c.) after termination of the mediumless stratification, to the required level as determined from their dry weight was burdoned by a considerable error. Thus the m.c. of the remaining samaras was each time checked after drying the samples at 105°C for 24 h.

Immediately after termination of the partial drying of the samaras they were subjected to a germination test in the same thermal regime as above and the rest of the samaras were placed in bottles, sealed and placed at −3°C for 12, 24, 30, 48

and 80 weeks, at 3°C for 12, 24 and 48 weeks. After termination of storage the seeds were subjected to a germination test in a sand-peat medium at 3°~25°C.

Results of the germinability of seeds stored for 0, 30 and 80 weeks at −3°C have been subjected to a computer variance analysis at $p=0.05$.

Table 1

Fraxinus excelsior L. The postulated and true moisture content of samaras partially dried after stratification without medium

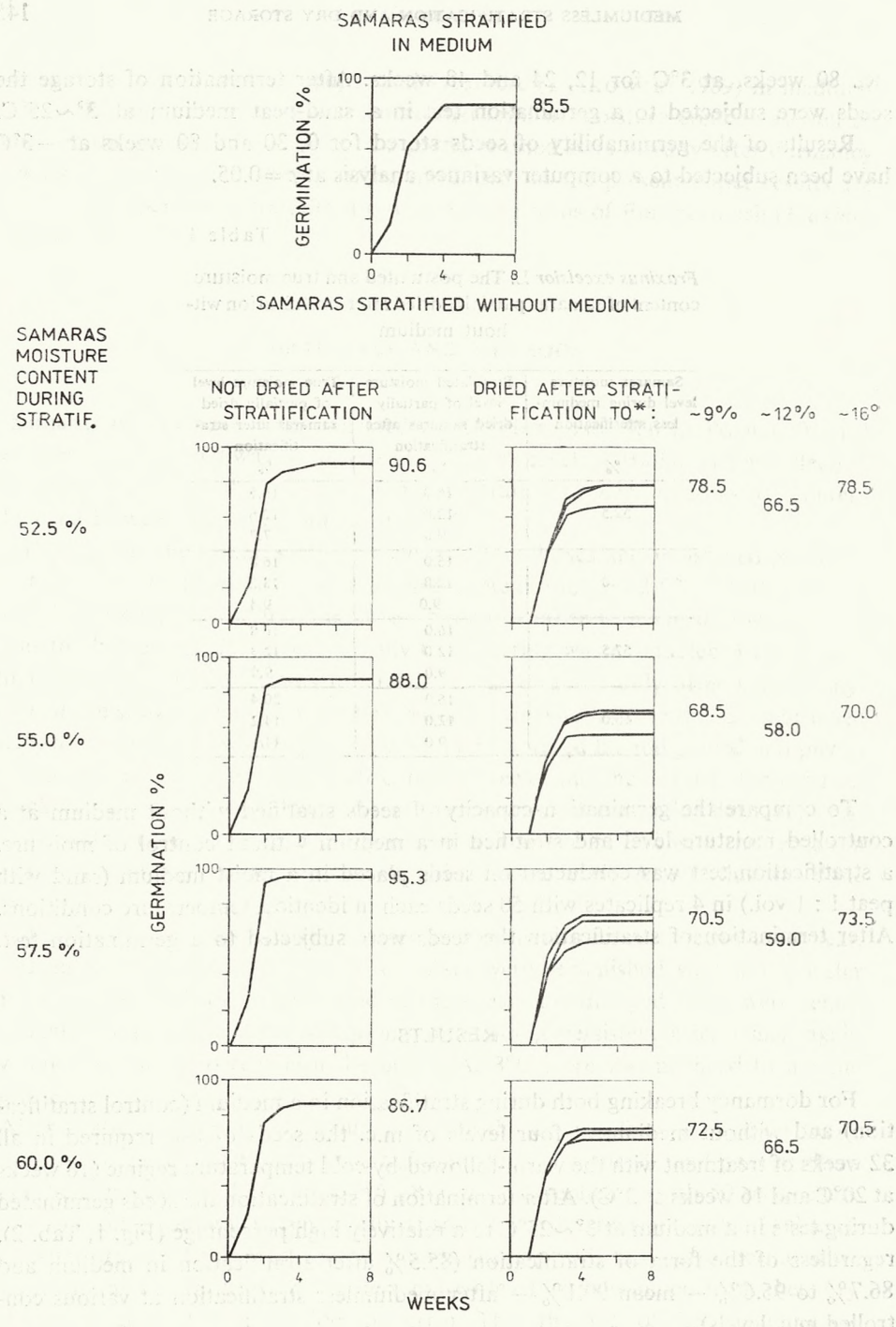
Samaras moisture level during mediumless stratification %	Postulated moisture level of partially dried samaras after stratification %	True moisture level of partially dried samaras after stratification %
52.5	16.0	16.5
	12.0	12.6
	9.0	7.7
55.0	16.0	16.8
	12.0	13.2
	9.0	9.4
57.5	16.0	16.8
	12.0	12.3
	9.0	9.9
60.0	16.0	20.4
	12.0	14.2
	9.0	11.1

To compare the germination capacity of seeds stratified without medium at a controlled moisture level and stratified in a medium without control of moisture, a stratification test was conducted on seeds placed in a moist medium (sand with peat 1 : 1 vol.) in 4 replicates with 50 seeds each in identical temperature conditions. After termination of stratification the seeds were subjected to a germination test.

RESULTS

For dormancy breaking both during stratification in a medium (control stratification) and without medium at four levels of m.c. the seeds of ash required in all 32 weeks of treatment with the warm-followed-by-cold temperature regime (16 weeks at 20°C and 16 weeks at 3°C). After termination of stratification the seeds germinated during tests in a medium at 3°~25°C to a relatively high percentage (Fig. 1, Tab. 2), regardless of the form of stratification (85.5% after stratification in medium and 86.7% to 95.6% − mean 90.1% − after mediumless stratification at various controlled m.c. levels).

Partial drying of the seeds/samaras after stratification without medium to three moisture levels (Tab. 1), has generally led to a lowering of germinability, on the average by about 17% when drying to ~16%, by 27% when drying to ~12% an by 47.6% when drying to ~9% m.c.



* TRUE MOISTURE LEVEL WAS GIVEN IN TABLE 1

Fig. 1. *Fraxinus excelsior* L. Course of germination of seeds during a germination test at 3°~25°C following stratification at 20°/3°C of whole samaras in a medium (control stratification) or without medium, moistened to 52.5%, 55.0%, 57.5% and 60.0%. After stratification the samaras were partially dried to ~9%, ~12% and ~16%

Seeds partially dried after stratification resumed germination immediately after being placed in a moist medium (sand with peat) during germination tests. About 1-week delay in the resumption of germination was presumably the time needed to absorb the water by the partially dried seeds (Fig. 1).

Table 2

Fraxinus excelsior L. Germination capacity (in %) of seeds during a germination test at 3°~25°C. Before the germination test the seeds were stratified mediumless in intact samaras at 4 moisture levels and partially dried after stratification to 3 levels and then stored in the partially dried state for 0, 30 or 80 weeks at −3°C

Samaras moisture content during mediumless stratif.	Postulated moisture level of samaras partially dried after stratif.*	Duration of storage of partially dried seeds (in weeks)		
%	%	0	30	80
52.5	9	78.5	75.5	73.5
	12	66.5	65.0	43.5
	16	78.5	58.5	35.5
55.0	9	68.5	75.5	74.0
	12	58.0	59.5	53.0
	16	68.5	44.4	43.5
57.5	9	70.5	79.0	68.0
	12	59.0	71.0	57.5
	16	73.5	48.5	39.5
60.0	9	72.5	72.5	40.5
	12	66.5	57.0	59.5
	16	70.5	68.5	45.5

* True moisture level was given in Table 1

Using the lowest level of m.c. for the samaras (52.5%) during stratification without medium has slightly increased the germinability of the seeds after their partial drying following stratification. Higher levels of m.c. of the samaras during stratification lowered by a few percent the germinability of the seeds after partial drying (Fig. 1). It is noteworthy that the seeds dried to 12% of m.c. had a markedly lower germination percentage.

Storage of European ash samaras for 48 weeks in sealed containers both at −3°C and at 3°C after stratification without medium and partial drying to ~9% (Tab. 3) did not lower the germination capacity compared to the non-stored seeds. On the other hand the level of germination was markedly lowered when the seeds were stored at both these temperatures following partial drying to ~12% and ~16% of m.c. At higher seed m.c. of the seeds stored at the higher temperature they were loosing their germinability more quickly (Fig. 2, Tab. 3). Even after storing seeds for 80 weeks (Tab. 2) at −3°C their germination capacity did not alter, (Tab. 2 and 4, Fig. 3 and 4) but this concerned only the seeds which have been stratified without medium at the two lower moisture levels (52.5% and 55.0%) and which

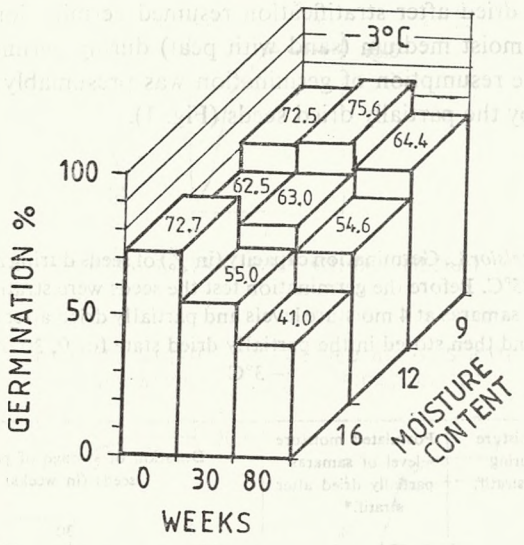


Fig. 2. *Fraxinus excelsior* L. Germination capacity of seeds during a germination test at 3°~25°C after stratification without medium (mean over 4 moisture levels) and partial drying to a moisture level of ~9%, ~12% and ~16% and storage for 0, 30 or 80 weeks at −3°C

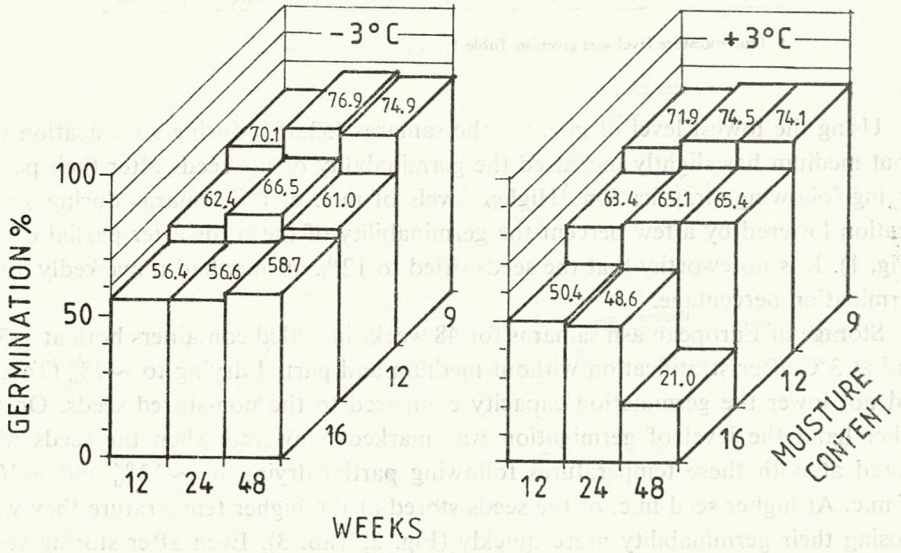


Fig. 3. *Fraxinus excelsior* L. Germination capacity of seeds during a germination test at 3°~25°C after stratification without medium (mean of 4 moisture levels) and partial drying to a moisture level of ~9%, ~12% and ~16% and storage for 12, 24 or 48 weeks at −3°C or 3°C

Table 3

Fraxinus excelsior L. Germination capacity (in %) of seeds during a germination test at 3°~25°C. Before the germination test the seeds were stratified mediumless in intact samaras at 4 moisture levels and partially dried after stratification to 3 moisture levels and then stored in the partially dried conditions for 12, 24 or 48 weeks at 3°C or −3°C

Samaras moisture content during mediumless stratif.	Postulated moisture level of samaras partially dried after stratif.*	Storage temperature	Duration of storage of partially dried seeds (in weeks)		
%	%	°C	12	24	48
52.5	9	3	77.5	76.0	76.0
		−3	74.5	80.5	77.5
	12	3	74.0	64.5	74.0
		−3	69.0	80.0	55.5
	16	3	54.0	39.0	9.5
		−3	68.0	58.0	48.0
55.0	9	3	70.5	78.0	75.0
		−3	73.5	77.0	79.0
	12	3	67.5	65.0	55.5
		−3	62.0	67.0	39.5
	16	3	55.0	47.5	23.5
		−3	45.0	48.5	74.0
57.5	9	3	74.0	73.0	72.5
		−3	67.0	73.0	71.0
	12	3	59.0	73.5	75.5
		−3	55.5	59.0	80.0
	16	3	46.0	45.0	10.5
		−3	51.0	57.0	44.5
60.0	9	3	65.6	71.0	73.0
		−3	66.5	77.0	72.0
	12	3	53.0	57.5	56.5
		−3	63.0	60.0	69.0
	16	3	46.5	63.0	40.5
		−3	61.5	63.0	68.5

* True moisture level was given in Table 1

Table 4

Analysis of variance for data summarized in Table 2

Source of variation	Sum of Squares	Degrees of freedom	Mean Square	F ratio	Significance level
Total	10798.282	143			
Initial moist (I)	109.3231	3	36.441	1.232	.3013
Final moist (F)	2031.6756	2	1015.838	34.337	.0000
Storage duration (S)	2607.0822	2	1303.541	44.062	.0000
2-Factor Interactions	2500.0799	16	156.255	5.282	.0000
I×F	786.2164	6	131.037	4.429	.0004
I×S	465.2714	6	77.545	2.621	.0201
F×S	1248.5920	4	312.148	10.551	.0000
Residual	3550.1217	136	48.786		

were partially dried after stratification to ~9% moisture level. Their germination capacity after storage was still high, 75.0% and 74.5% respectively. In the other variants of the experiment the germination capacity of the seeds was more or less lower.

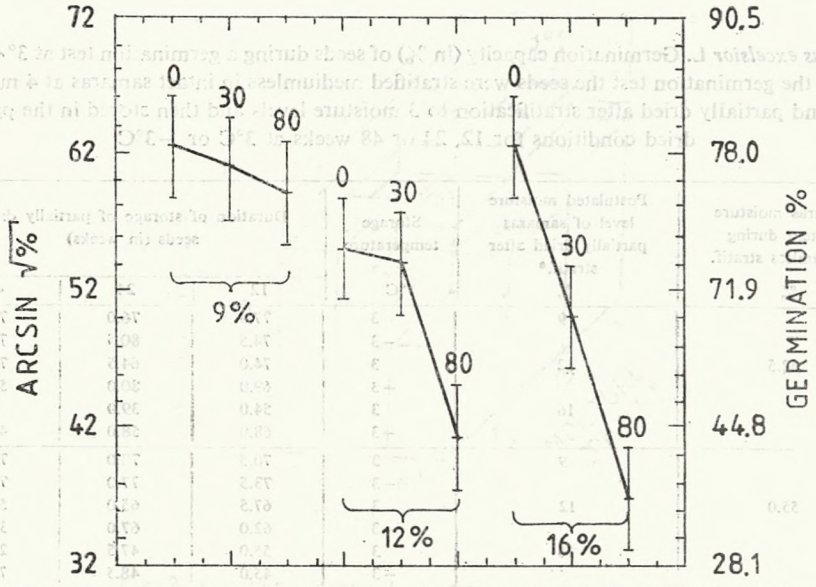


Fig. 4. *Fraxinus excelsior* L. Germinability of seeds stratified without medium (20°/3°C, 16+16 weeks) at 52.5% of moisture content followed by partial drying to ~9%, ~12% or ~16% m.c. and stored at ~3°C for 0, 30 and 80 weeks

DISCUSSION

Storage of after-ripened seeds of European ash by their partial drying is an alternative way of withholding their germination. The other method is to lower the temperature below 0°C (Suszka 1987).

In a literature review concerning the possibility of storage through partial drying of seeds deprived of dormancy (Tylkowski 1988, 1989) methods were presented and the possibilities of using them for a number of species both coniferous and broadleaf. It is worth mentioning however that seeds of these tree species require for the removal of dormancy only a cold treatment during stratification (sometimes preceded by a short warm phase). In the case of European ash from Poland and from a border zone with USSR the seeds require a long, lasting 16 weeks warm stratification at 15 - 20°C preceding the cold stratification of similar duration (Suszka 1987, Nikolaeva and Vorobieva 1978, Tylkowski 1988). During the warm phase of stratification without medium there exists a real and considerable danger of fungal infection, particularily since the high temperature and moisture level favour the development of fungi, not only saprophytic ones but also parasitic. For this reason it is necessary to employ fungicides during this phase. It appears that the fungicides should be used preventively, that is shortly after the beginning of moisteining samaras, and not only after first molds are observed. The 1% Benlate solution used in the trial proved harmless to the seeds and at the same time very effective in combating the appearing fungi. The preventive use of this fungicide may prove to be sufficiently effective to reduce its dose during mediumless stratification.

During warm-followed-by-cold stratification in a stratification medium the use of the fungicide proved unnecessary. Generally no significant difference was observed in the germination capacity after mediumless stratification at four levels of samaras m.c. (Tab. 4). These differences however became manifest after partially drying the samaras to various moisture levels following dormancy breaking. The most satisfactory results were obtained after stratification of the samaras held at a moisture level of 52.5% and 55.0% during warm-followed-by-cold stratification and after storage following partial drying of the after-ripened seeds to ~9% m.c. (Tab. 2, Fig. 4). Storage of non-dormant samaras at the higher m.c. levels led to a lowering of germinability the more so the longer was the storage.

Lack of significant differences in germinability of partially dried seed to ~9% after mediumless stratification and storage for 48 weeks at 3°C or −3°C justifies rejection of the below zero storage temperature for seeds of European ash. Unfortunately in the trial the variant with 3°C was not employed for the longest (80 weeks) period of storage, basing on the assumption that a 48 week period is sufficiently long and that a longer storage should be conducted at a below zero temperature which was tested. After storage for 80 weeks at −3°C the seeds partially dried to ~9% of m.c. maintained their germinability at a level equal to the original one prior to storage (Tab. 2). The results reported here should however be confirmed by studies on broader seed material.

LITERATURE

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Stratyfikacja bez podłoża i przechowywanie niespoczynkowych nasion *Fraxinus excelsior* L.

Streszczenie

Podsuszone po zbiorze i krótko przechowywane skrzydlaki jesiona wyniosłego nawilżano do 52,5%, 55,0%, 57,5% i 60,0%. Wilgotność skrzydlaków utrzymywano na stałym poziomie początkowo przez 16 tygodni w 20°C, a następnie przez dalsze 16 tygodni w 3°C, aż do początku

kiełkowania nasion. Po łącznie 32 tygodniach stratyfikacji bez podłoża skrzydlaki podsuszono do ~9%, ~12% lub ~16% wilgotności i przechowywano w szczelnie zamkniętych butelkach w 3° lub −3°C. Po 48 tygodniach przechowywania w obu temperaturach oraz po 80 tygodniach przechowywania w −3°C, nasiona podsuszone do ~9% kiełkowały podczas prób kiełkowania w 3°~25°C natychmiast po napęcznieniu, a ich zdolność kiełkowania pozostawała na poziomie odpowiadającym początkowej, wysokiej zdolności ich kiełkowania (około 75%).