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# After-ripening of European ash (*Fraxinus excelsior* L.) seeds matured in dry weather conditions

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*Abstract*. An effective method was elaborated for a presowing treatment of European ash seeds, ripened during a drought. Dormancy of such seeds is not overcome by stratification in a moist medium nor without it at a controlled moisture content of samaras (55%).

The method bases on periodically repeated soaking of samaras in water (18°C) for 1 hour and after that draining them off and keeping in wet conditions without medium at 15°/3°C or 20°/3°C (16+16 weeks). Periodic soaking of samaras proved effective when repeated in the warm phase once a week and in the cold phase every second week. After such treatment seedling emergence in the nursery is energetic and in high percent.

*Additional key words*: dormancy, drought, stratification, leakage, germination.

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## INTRODUCTION

In Poland, under natural conditions, fully ripe European ash seeds start to germinate and seedlings emerge after long lasting action of successive periods of both warm and cold temperature in the moist soil. After autumn sowing of ripe not-stratified samaras, seedlings emerge usually in the second spring. In order to overcome dormancy in controlled conditions, seeds should also be stratified in two, warm-followed-by-cold steps (Varasova 1956, Judin 1973, Suszka 1978). During the warm phase of stratification at 15–20°C for 14–16 weeks, undeveloped embryos, with a mean embryo-index of 50–60%, enlarge their size, mainly through cell elongation. At the end of this period one can observe first cell divisions, and their number increases (on longitudinal section) from 180–200 to 400–450 i.e. 2-2.5 times (Ljašuk 1971). Further embryo growth continues in the cold phase (3°C) lasting 16 weeks, until an embryo-index above 90% is achieved.

Dormancy of European ash seeds collected in years with normal weather conditions, can be overcome by treating them without any medium (naked stratification) at a controlled moistrue content of samaras (Tylkowski 1990). So treated samaras with a moisture content of 52.5–60.0%, held at 20°/3°C, germinated 1.2–10.2% more than during standard stratification in a medium at the same temperatures.

Both the above mentioned methods of breaking dormancy of European ash seeds, collected in the exceptionally dry year 1989, were insufficiently effective and it became necessary to find another method of breaking dormancy of these seeds.

## MATERIALS AND METHODS

The samaras were collected on 24th October 1989, in Poznań, from three individual trees. After drying they were stored at −3°C in sealed containers. The characteristics of this seed material after storage are presented in Table 1.

Table 1

*Fraxinus excelsior* L. Seed characteristics after storage.

| | | Tree no. | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Moisture content of samaras | % | 10.4 | 10.7 | 12.6 |
| Moisture content of seeds | % | 9.4 | 9.7 | 9.7 |
| Seed viability (TTC) | | | | |
| viable seeds | % | 96.5 | 95.5 | 91.0 |
| dead seeds | % | 3.5 | 4.5 | 9.0 |
| Embryo index | % | 63.2 | 54.7 | 62.1 |
| Period of storage at −3°C | days | 478 | 478 | 478 |

### Thermal conditions of breaking dormancy

Seeds, gathered separately from each of the three trees, remaining in samaras, were treated at 15°/3°C or 20°/3°C, each of the two thermal phases lasting 16 weeks.

### Embryo index

Measurements of embryos and the corresponding, longitudinally cut, seeds were performed under microscope in 3 replicates, each with 10 seeds. Embryo index i.e., the proportion of the embryo length to the seed length is expressed as a mean percentage value.

**Stratification in the medium**

A moist mixture of sand and peat (1:1 by vol.) was used as the stratification medium. Humidity of this mixture was checked weekly in the warm phase of the warm-followed-by-cold stratification and every two weeks in the cold phase. Water losses were replenished.

**Pretreatment without medium at a moisture content of 55%**

Intact samaras, separately for each tree, were moistened in plastic boxes with tap water to a moisture content of 55%, relative to fresh weight. The moistening was performed by repeated spraying and mixing of samaras for 3 days, avoiding water collecting at the bottom of the boxes. After achieving the planned moisture level, it was held constant by controlling it once a week in the warm phase and every two weeks in the cold phase (Tylkowski 1990).

**Pretreatment without medium by cyclically repeated soaking of samaras in water**

The samaras were placed (separately for each tree) into plastic boxes and submerged in water. The samaras were immersed in water for 1 hour, mixed several times, and after that the water was poured out. Such periodical soaking of samaras in water was repeated every week during the warm phase and every two weeks in the cold phase. After each soaking, the boxes with wet samaras were covered by a lid and placed in adequate temperature.

**Germination tests**

After the warm-followed-by-cold pretreatments, the samaras (4 replicates with 50 or 40 seeds each) were mixed with the standard stratification medium. The germination tests were then carried out at an alternating temperature 3°~25°C (16+8 hours/day). Seeds with radicles at least 3 mm long were recognized as germinating seeds.

**Nursery seedling emergence**

Only samaras held without medium in the warm-followed-by-cold system (15°/3°C or 20°/3°C, 16+16 weeks) and cyclically soaked in water, were sown in the nursery on 30th March, 1992. Seedling emergence was checked on 1st June of the same year. Samaras (4 × 50 seeds) were sown in rows, pressed into the soil 2 cm deep, then they were covered with soil and a 10 cm layer of straw, which was removed when the first seedlings emerged.

## RESULTS

Values of the embryo index, depending on the treatment methods and on the after-ripening thermal conditions, are listed in Table 2. During stratification in both systems i.e., in the medium and without any medium, at a regulated moisture content of samaras of 55%, and in both thermal systems, the embryo index increased to 58.1–71.3% after the warm phase, and to 66.9–81.8% after the cold one. Embryo index of seeds held without medium and cyclically soaked in water differed considerably from the embryo index of seeds stratified in the medium or moisturized to 55%. It reached 70.9–83.9% after the warm phase and 90.0–93.2% after the cold one.

Table 2

*Fraxinus excelsior* L. Values of the embryo index (in %) presented separately for the mother trees, methods of treatment and temperature conditions for after-ripening. Measurements were performed after the warm and cold phases of the warm-followed-by-cold treatment (16+16 weeks).

| Tree no. | Treatments | Temperature conditions of after-ripening | Weeks of warm phase | Weeks of cold phase |
|---|---|---|---|---|
| | | | 16 | 16 |
| 1 | Stratification in medium | 15°/3°C | 65.6 | 71.5 |
| | | 20°/3°C | 66.5 | 75.2 |
| | Pretreatment at controlled moisture content | 15°/3°C | 68.2 | 70.8 |
| | | 20°/3°C | 63.6 | 72.7 |
| | Pretreatment with cyclical soaking | 15°/3°C | 83.2 | 92.7 |
| | | 20°/3°C | 76.4 | 92.5 |
| 2 | Stratification in medium | 15°/3°C | 64.1 | 72.6 |
| | | 20°/3°C | 61.6 | 77.0 |
| | Pretreatment at controlled moisture content | 15°/3°C | 58.1 | 66.9 |
| | | 20°/3°C | 60.7 | 77.8 |
| | Pretreatment with cyclical soaking | 15°/3°C | 74.1 | 93.2 |
| | | 20°/3°C | 70.9 | 93.0 |
| 3 | Stratification in medium | 15°/3°C | 71.3 | 81.8 |
| | | 20°/3°C | 67.8 | 78.3 |
| | Pretreatment at controlled moisture content | 15°/3°C | 67.6 | 70.4 |
| | | 20°/3°C | 65.8 | 67.7 |
| | Pretreatment with cyclical soaking | 15°/3°C | 83.9 | 90.0 |
| | | 20°/3°C | 70.3 | 90.6 |

The size of the embryo index was adequate for germinative capacity of seeds pretreated by all three methods (Table 3).

Table 3

*Fraxinus excelsior* L. Germinative capacity of samaras (in %) during germination test at 3°~25°C after stratification in a medium or after pretreatment without medium with regulated (55%) moisture content or with cyclical soaking in water. In brackets there is nursery seedling emergence in %.

| Tree no. | Germinative capacity in % | | | | | |
|---|---|---|---|---|---|---|
| | Stratification in medium | | Pretreatment with regulated moisture content 55% | | Pretreatment with cyclical soaking in water | |
| | 15°/3°C | 20°/3°C | 15°/3°C | 20°/3°C | 15°/3°C | 20°/3°C |
| 1 | 17.5 | 33.1 | 34.0 | 7.5 | 78.0 [86.0] | 83.5 [70.5] |
| 2 | 33.7 | 29.3 | 59.5 | 15.6 | 89.5 [89.0] | 94.5 [85.5] |
| 3 | 34.0 | 16.2 | 8.1 | 3.7 | 90.5 [76.0] | 71.5 [47.5] |

After stratification of samaras in the medium, the germinative capacity of seeds remained at a relatively low level of 16.2–34.0%. Seeds from trees 2 and 3, stratified at 15°/3°C, germinated to a higher percentage than those stratified at 20°/3°C, but seeds from tree 1 have responded in the opposite way (Table 3).

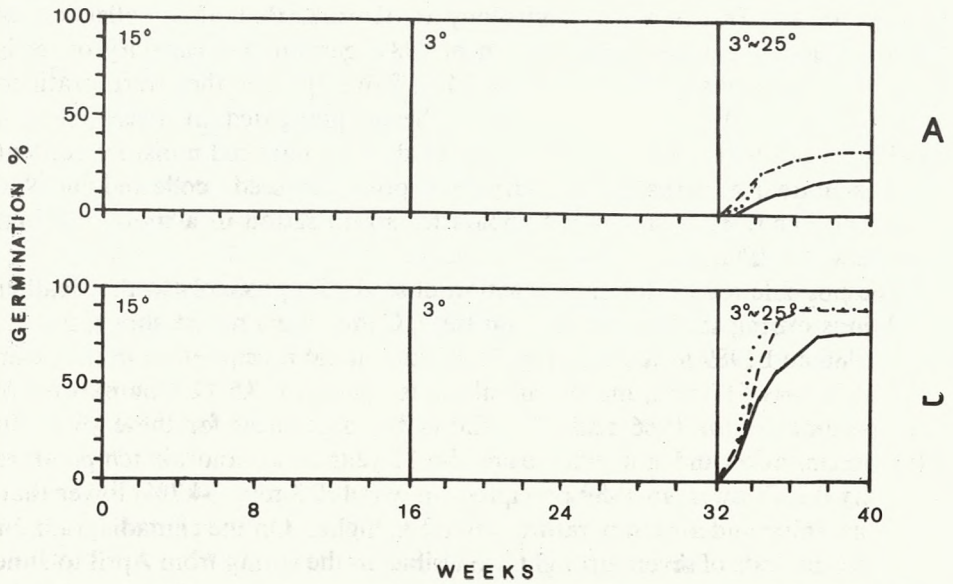


Fig. 1. *Fraxinus excelsior* L. Course of seed germination (in a peat/sand medium), from 3 different trees during germination test at 3°~25°C, conducted after stratification of samaras in medium (A) or after treatment without medium with cyclical soaking of samaras in water (B). Continuous line – tree no. 1; broken line – tree no. 2; dotted line – tree no. 3.

Seeds in samaras pretreated without any medium at a controlled moisture content of 55%, have germinated to a similar low level (3.7–34.0%), with the exception for seeds from tree 2, which germinated in 59.5%, when treated at 15°/3°C. In general, seeds held without medium at 15°/3°C germinated better than after treatment at 20°/3°C.

After cyclically repeated soaking of samaras in water, the germinative capacity of seeds increased in both the applied thermal systems to 71.5–94.5%. In the germination test at 3°~25°C, seeds germinated very energetically (Fig. 1). Only slight differences were observed in the germinative capacity of seeds from trees 1 and 2 after pretreatment in the thermal systems. Seeds from tree 3 germinated higher after pretreatment at 15°/3°C than at 20°/3°C.

In the nursery seeds have emerged at a similarly high level as in the laboratory germination tests at 3°~25°C. After pretreatment at 15°/3°C, 76.0-89.0% seedlings have emerged, and after 20°/3°C treatment 47.0-85.5% (Table 3).

## DISCUSSION

It was found during investigations carried out at the Department of Seed Biology of the Institute of Dendrology in Kórnik, that after collection of European ash samaras in the autumn of 1989, germinative capacity of seeds was at an exceptionally low level (max. 34%, Table 3), when they were stratified at 15°/3°C or 20°/3°C (16+16 weeks). Seeds pretreated at these thermal conditions but without any medium and held at a controlled moisture content (55%, samaras) germinated similarly. European ash seeds collected in 1986 (from another tree) germinated 85.5% after stratification in a moist medium (Tylkowski 1990).

The possible reason for such reaction of seeds lies probably in the weather conditions during seed maturation on trees. Climadiagrams are shown for the years 1986 and 1989 in Kórnik (Fig. 2). Mean annual precipitation in the years 1981–1991 was 517 mm, mean annual air temperature 8.5°C. Comparison of both parameters for 1986 and 1989 shows big differences for these years. In 1986 precipitation did not differ from the 11-year mean and air temperature was only 0.7°C lower. In 1989 precipitation was 179.3 mm (34.7%) lower than the mean value and air temperature was 1.9°C higher. On the climadiagram for 1989, two periods of severe drought are visible, in the spring from April to June and at the turn of summer and autumn, from August to October. In the summer months there was also a dry spell, which means that in consequence the whole vegetative season was characterized by a deep deficiency of water accessible to plants. In 1986 there was no water deficit.

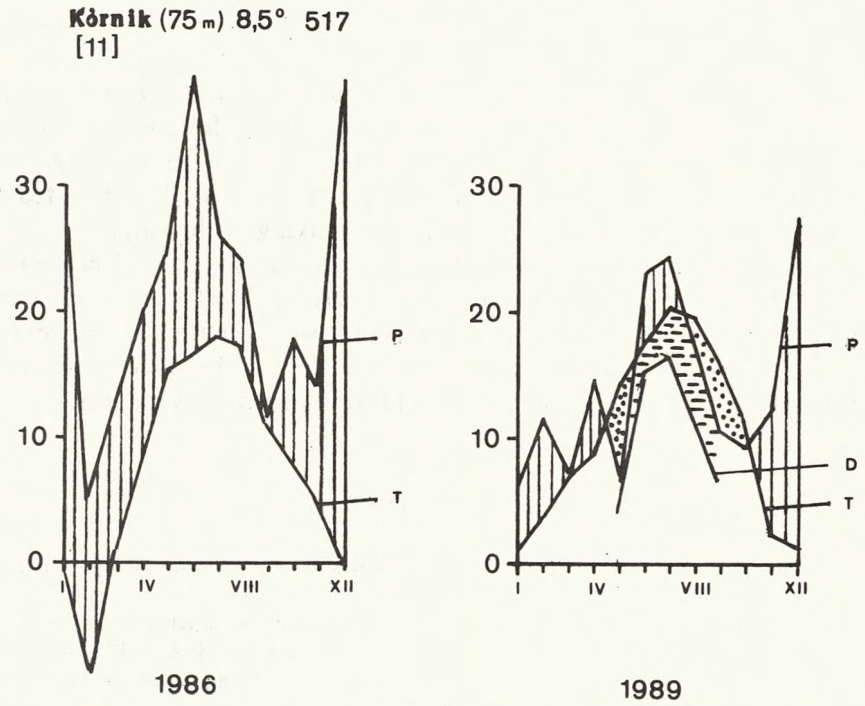


Fig. 2. Climadiagrams for Kórnik for 1986 and 1989. Kórnik lies at an altitude of 75 m. On the basis of 11-year observations, the mean annual temperature is 8.5°C and mean annual precipitation is 517 mm. T – temperature; P – precipitation; D – precipitation on a different scale; dotted area – drought period; horizontal-lined area – dry spell; vertical-lined area – relatively wet period. Scale on ordinate; degrees (°C) and precipitation in mm × 2 for P and mm × 3 for D.

Cyclically repeated soaking of whole samaras during pretreatment without any medium with a warm-followed-by-cold 15°/3°C or 20°/3°C (16 + 16 weeks), thermal regime resulted in the breaking of dormancy of most seeds (Table 3). Soaking of samaras for 1 hour repeated once a week in the warm phase and once every second week in the cold phase, sufficed to overcome seed dormancy. Already after the warm phase of pretreatment larger increase of embryo length was observed when the seeds were soaked than stratified. It is possible that during the repeated soaking different substances inhibitive to the after-ripening process were leached out from the samaras into the water. In the first weeks of the cyclical soaking of samaras an intensive brown colour of the water was observed after 1 hour of the water bath. Neither during stratification in a medium nor during pretreatment without any medium at a controlled moisture content (55%) during maturation of samaras on trees, were these substances leached out in sufficient quantity to induce germination. It seems that rains leach some of the inhibitory substances from the samaras when they are still on the trees. In this way we can explain the elevated germinative

capacity of seeds collected in 1986, after a pretreatment without medium at a regulated moisture content in the range of 52.5–60.0% (Tylkowski 1990).

Occurrence and inhibiting effect of germination inhibitors in European ash seeds were found in the pericarp (Wciślińska 1977), Rypák 1978/1979), in the endosperm (Kentzer 1966) and in the embryo (Villiers and Wareing 1960). The latter authors have also found that embryo axes isolated from dormant seed can grow after 48 hours of soaking with water.

The study shows that the cyclical soaking in water of European ash samaras, during their presowing treatment, increases its efficacy. This concerns especially seeds maturing in a period of severe water deficiency. However, the efficacy of this method should be still tested on a broader seed material collected in different years and representing various provenances.

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## Ustępowanie spoczynku nasion jesiona wyniosłego (*Fraxinus excelsior* L.) dojrzewających w warunkach suszy

## STRESZCZENIE

Opracowano metodę przezwyciężania spoczynku nasion jesiona wyniosłego (*Fraxinus excelsior* L.), dojrzewających w warunkach silnej suszy, której towarzyszyło oddziaływanie temperatur wyższych od przeciętnych.

Po standardowej stratyfikacji w podłożu lub stratyfikacji bez podłoża, przy regulowanej wilgotności skrzydlaków 55%, w układzie cieplnym 15°/3°C (16+16 tyg.), nasiona kiełkowały podczas prób kiełkowania w 3°~25°C w wyjątkowo niskim procencie (śr. 24.4%). Wymienione układy cieplne zapewniają zazwyczaj skiełkowanie i wzejście większości nasion.

Zastosowanie cyklicznie powtarzanego moczenia skrzydlaków przez 1 godzinę w wodzie wodociągowej o temp. 18°C, w fazie ciepłej co tydzień, a w fazie chłodnej co 2 tygodnie, i po odsączeniu wody, pozostawienie ich bez podłoża (w stanie wilgotnym) w takich samych warunkach cieplnych jak podczas stratyfikacji w podłożu, przyczyniło się do skiełkowania nasion, dojrzewających w warunkach wyżej scharakteryzowanych, w bardzo wysokim procencie (śr. 84.6%). Po wysiewie w szkółce nasiona te wzeszły energicznie w 78.5%.