Management of Seed Dormancy in *Fagus sylvatica, Fraxinus excelsior,* and *Prunus avium*

Andrea Nowag
Hinterm Holz 1, Kirchhatten, Germany

INTRODUCTION

This paper reviews results from one element of a 3-year European Union (EU) funded project, *A multidisciplinary approach to the understanding and efficient handling of seed dormancy in tree species* (October 1993 to October 1996). It included work in Denmark (coordinator, four research groups), Great Britain (two research groups), The Netherlands, France (five research groups), Spain, and Germany. It was divided into two groups. One group worked with applied technology and biology with regard to harvest, dormancy breaking treatments, and storage of pretreated seeds. The other studied the physiological and biochemical processes involved in seed dormancy. This review covers the main results of the applied group on the species *Fagus sylvatica, Fraxinus excelsior*, and *Prunus avium.*

Some 60% of the tree species from the temperate region, particularly hardwood species, have seeds which can be regarded as deeply dormant. This prevents germination until the dormancy has been released. It is known that the seed is dormant at maturity, but the timing of the onset of dormancy and the factors influencing it are still under research. There are different kinds and combinations of dormancy. Embryo dormancy is the most common kind in tree seeds from the temperate regions (Bewley and Black, 1994).

Embryo dormancy can be released by stratification treatment at low temperature and at high moisture content, a well known procedure used successfully for many years. The term "stratification" today also includes all treatments, not just cold, to release seed dormancy. There are however problems with management of stratification methods used on nurseries. For example, depending on environmental conditions the optimum cold stratification period varies from year to year. Stratification requirement can also vary within a seed lot. Another problem is that seeds can germinate at the stratification treatment temperature and in some cases up to 20% of the seeds in a batch may germinate during stratification. Existing stratification methods for tree seeds are time consuming, inflexible, and unreliable. At least 30% of the potentially productive seeds in a batch fail to germinate due to ineffective stratification methods.

The objectives of the applied element of the EU funded work were, therefore:

- To obtain a better understanding of the seed dormancy process including all phases from dormancy induction to release, combining basic and applied seed research for an overall view.
- To improve seed handling procedures for dormant tree seeds with particular emphasis on the development of more efficient and versatile stratification methods.
- To improve viability tests of dormant seeds and investigate whether it will be possible to determine the level and state of dormancy.

The project was divided into seven connected tasks:

1) Optimal seed collection and handling with regard to dormancy level, pretreatment, and storage (studied on *Fagus sylvatica*, *Fraxinus excelsior*, *Prunus avium*, *Sorbus mougeotii*, *Malus sargentii*, and *M. sieboldii*).
2) The effects of pretreatment conditions on the efficiency of dormancy breaking and laboratory assessment of dormant tree seeds (studied on *F. sylvatica*, *F. excelsior*, *P. avium*, *Pseudotsuga menziesii*, *Acer platanoides*, and *A. pseudoplatanus*).
3) Dormancy breaking by exogenous application of hormones (studied on *F. sylvatica* and *P. menziesii*).
4) Redrying and storage of pretreated seeds (studied on *F. sylvatica, F. excelsior, P. avium,* and *P. menziesii*).
5) Endogenous hormones involved in seed dormancy (studied on *F. sylvatica* and *P. menziesii*).
6) Molecular changes during dormancy induction and dormancy breaking (studied on *F. sylvatica* and *P. menziesii*).
7) Production of a tree seed dormancy database.

COMMON BEECH (*Fagus sylvatica* L.).

Beechnuts have a very deep and heterogenous dormancy. The dormancy varies from year to year, from seedlot to seedlot, and among seeds within the same seed lot. The seeds are dormant at collection and thus fail to germinate in conditions under which nondormant seeds germinate rapidly.

Optimal Seed Collection and Handling.

Collection. Seeds were harvested from two provenances on three occasions (Weeks 35, 38, and 44) during 1993. The seeds started to shed about week 38. The first two collections were made from the trees, the last from the ground (Thomsen, 1997). Both seed sources were fully germinable at the first harvest. One provenance had more mature seeds than the other, indicated by a lower moisture content (44% and 40% respectively, fresh weight basis). Seeds harvested early in development could germinate, but only at low temperatures. The more mature the seeds were, the faster they germinated. The mean germination time (MGT) was about 22 weeks for the first harvest, about 18 weeks for the second, and about 13 to 14 weeks for the last harvest. These results indicate that the seeds are "born" dormant and it is not possible to overcome dormancy by collecting the seeds before maturity. However, if the seeds are left too long on the ground they may deteriorate. The optimal time of collection is therefore as soon after natural shedding as possible. In this investigation, the optimal collection time was Week 38 for the provenance with the lowest moisture content (more mature seeds) at the first harvest. For the second provenance the optimal collection time was Week 44 (Thomsen, 1997).

Handling. Beechnuts from all three collections were dried at three different rates: (1) at 15C and 15% relative humidity (RH) to about 8% moisture content (fresh weight basis); (B) at 20C and 15% RH 4 h day to 8% moisture content; (C) at 20C and 15% RH 8 h day to 20%, 16%, 12%, and 8% moisture content.

Germination tests were performed before and after drying and after 10 and 16 months subsequent storage at 5C (Thomsen, 1997). Drying to 8% moisture content reduced the mean germination time (MGT) by about 3 weeks. In beechnuts, drying can, therefore, substitute for part of the cold stratification. Drying rates had no effect on the speed of germination. However, there seems to be a relationship between moisture content and MGT: drier seeds germinated more rapidly than moister seeds (Thomsen, 1997). There was no significant difference in survival following drying to 8% moisture content under the different drying rates except in the first harvest of one provenance. Here the faster rates resulted in more damage (Thomsen, 1997). Beechnuts dried to 8% moisture content stored well at 5C, with only slight decreases in germination capacity. The best short- and long-time survival was obtained by drying the seeds at 15C and 15% RH. When comparing the two drying treatments at 20C it was found that the faster rate was more damaging than the slower (Thomsen, 1997).

Pretreatment Conditions. In beechnuts cold stratification breaks both embryo dormancy and seed-coat-imposed dormancy and allows the seeds to germinate at a wider range of temperatures and increases the germination rate (Derkx and Joustra, 1997).

Beechnuts from three provenances (The Netherlands, Poland, and Denmark), with a moisture content of 30%, were stratified at 3C for varying periods from 4 to 60 weeks. Germination at a range of temperatures was tested after the pretreatment. No germination occurred during stratification. Freshly harvested seeds hardly germinated at 10C or above. Stratification for between 16 to 20 weeks increased the range of temperatures over which germination occurred, so that the seeds could germinate between temperatures of 0 to 20C. Stratification for up to 24 weeks decreased the mean germination time, but stratification for longer periods increased MGT (Derkx and Joustra, 1997). The optimum cold stratification period for fresh beechnuts from the three provenances tested was 16 to 20 weeks (Derkx and Joustra, 1997) with 20 weeks generally giving optimal dormancy breakage, although shorter durations may give good emergence in the field (Derkx, 1996). Increasing the temperature range of germination has the important implication that temperature conditions at the time of sowing in spring become much less critical (Derkx and Joustra, 1997). For practical purposes the optimal duration of stratification for dormancy breakage coincides with that for increasing the germination temperature range (Derkx and Joustra, 1997). Premature germination during a dormancy releasing treatment can be prevented by controlling the moisture content. The moisture content of beechnuts should be a maximum of 32% (based on fresh weight).

Redrying and Storage of Pretreated Seeds. After 8, 12, and 16 weeks pretreatment, portions of the seeds from all three provenances from the trial described above were transferred to storage conditions. They were either stored without drying or dried to moisture contents of 9% or 16%. The moisture content of 9% was obtained by drying for 7 days at 17C and 45% RH. The moisture content of 16% was obtained by drying for 7 days at 17C and 75% RH. The seeds were stored at -2 or +3C in perforated bags. Germination was tested in the dark at a range of temperatures of 3, 10, 15, 17, 20, 25, and 30C (Derkx and Joustra, 1997).

Seeds stratified for 8 weeks and stored undried at -2C for a further 8 weeks showed a remarkable increase in germination. A positive effect on germination

was also observed when undried, 8-week-stratified seeds were stored at 3C (Derkx and Joustra, 1997).

Dutch seeds stratified for 12 weeks and stored with moisture contents of 9% or 16% at -2C for 8 weeks showed a large reduction in germination capacity at germination temperatures above 20C. Storage at 3C of seeds from the same pretreatment length and drying regime reduced germination even more and under these conditions the drier seeds suffered more. Seeds from the Polish and Danish sources were even more affected by these treatments (Derkx and Joustra, 1997).

The effects of dehydration and dry storage depended on the duration of the dormancy-breaking pretreatment. Increasing the duration of pretreatment from 8 weeks to 12 or 16 weeks reduced desiccation tolerance and storability of the seeds. It may be hypothesised that dormancy breakage is complete after a certain period of pretreatment, and following this, early germinative events preparing the seeds for radicle protrusion may start, since the moisture content of the seeds (30%) during stratification allows metabolic activity. A proposal is that seeds that are dehydrated during the phase of dormancy breakage withstand dehydration and dry storage, whereas dehydration during the phase of early germination events causes irreparable damage (Derkx and Joustra, 1997).

It follows that if the seeds are to be redried and stored, the pretreatment duration should be shortened. After pretreatment of 7 to 10 weeks the seeds can be dried to a moisture content of 8% to 10% and be stored at -2C for at least 6 months without loss of germinability. The dormancy breaking and germination temperature effects of the pretreatment are maintained during storage (Derkx, 1996).

Application of Hormones. Pretreatment duration can be reduced by application of gibberellins on naked *F. sylvatica* seeds. Application of gibberellin on whole seeds had limited effect (Corbineau et al., 1995).

Seeds treated with ethylene showed a distinct increase in the endogenous gibberellins GA_3 and GA_{19}. Possibly the promoting effect of ethylene on dormancy breaking is through increasing gibberellin concentrations. The ethylene-releasing compound ethephon is normally used to apply ethylene conveniently. Treatment with ethephon on whole seeds showed a distinct increase in germination. By combining cold treatment and ethephon treatment, the duration of pretreatment could be reduced by 50% (Corbineau et al., 1995; Falleri et al., 1997).

Conclusions.

- The optimal collection time of *F. sylvatica* seeds is as soon after seed shed as possible.
- Cold stratification at about 5C for 16 to 24 weeks gives optimal dormancy breakage for northern Europe provenances.
- Cold stratification of 16 to 24 weeks widens the germination temperature range so that seeds germinate between 0 and 20C.
- Premature germination during stratification can be prevented by controlling the moisture content and adjusting it to maximum 32%.
- For storage of stratified seeds the stratification period should be shortened to 7 to 10 weeks.
- For storage the seeds can be dried to a moisture content of 8% to 10% and be stored at -2C for at least 6 months without loss of germinability.

COMMON ASH (*Fraxinus excelsior* L.)

Ash fruits have an underdeveloped embryo when the fruits fall from the tree in autumn. For full development of the embryo a warm treatment is needed. For dormancy release a cold treatment is needed. The warm treatment usually precedes the cold.

Optimal Seed Collection and Handling.

Optimal Seed Collection. Ash fruits from six different trees in the U.K. were collected on five occasions. The fruits were pretreated in a peat and sand (1 : 1, v:v) medium for 16 weeks at 15C followed by 16 weeks at 4C. Embryo development and optimum germination temperatures were examined. The seeds were germinated at a range of constant temperatures from 3.5 to 25C and at the alternating temperatures of 5/15 and 5/25C (12h/12h) (Jones and Gosling, 1996).

The collection date did not influence the rate of embryo growth during stratification. Significant differences between the trees were found concerning the embryo lengths. The optimum germination temperature was between 3.5 and 10C. At higher temperatures germination decreased, probably because of the induction of secondary dormancy. For batches in which dormancy had not been completely broken, germination was better under alternating temperatures (Jones and Gosling, 1996).

Pretreatment Conditions. During the warm phase of pretreatment full development of the embryo appears to depend on the presence of some kind of medium around the seeds. The rate of embryo growth was influenced by the composition of the medium, with peat-based media performing better than vermiculite and sand. Pericarp degradation was much greater in fruits stratified in a medium suggesting that embryo growth is inhibited by the pericarp (Derkx, 1996).

Washing the fruits in running water for 48 h before pretreatment effectively improved embryo growth whether or not the pretreatment occurred in the presence of a medium. The washing probably removed a soluble growth inhibitor in the fruits. Washing the fruits, followed by pre-treatment including 4 to 8 weeks of warm period without medium, increased germination compared with unwashed seeds pretreated in the presence of a medium.

Stratification of washed ash fruits without any medium offers more flexibility in dormancy breakage procedures. The warm phase could be shortened to 4 weeks, but 8 weeks ensures consistently better germination (Jones and Gosling, 1996).

The optimum temperature for embryo elongation in the warm stratification phase was 15C, though 10C or 20C also were suitable. However, seeds pretreated at 20C were less likely to germinate during the following cold phase than those pretreated at 15C. Embryos did not elongate during cold treatment at 5C unless they had received a warm stratification phase first; however, embryos do continue to grow at 5C once they have received at least 4 weeks warm stratification. The duration of the cold phase had a clear effect on the MGT. The stratification should consist of 4 months at 15 to 20C and be followed by at least 16 weeks of cold treatment at 3C. A period of 24 weeks of cold treatment gave significantly more germination (Derkx, 1996).

Embryo growth was unaffected by fruit moisture contents between 45% to 60% (fresh weight basis). However, at 60% moisture content, germination was reduced.

Germination was not affected by moisture contents between 45% and 55%. Moisture content between 50% to 55% turned out to be too high to prevent premature germination during the cold phase. A moisture content of 45% is ideal (Derkx 1996).

Redrying and Storage of Pretreated Seeds. Full stratification of freshly harvested fruits in a medium, followed by redrying and storage for 3 months, resulted in only a small reduction of germination capacity. Drying after the warm phase only caused large decreases in germination capacity (Derkx, 1996).

Conclusions.

- The collection date (after shedding) does not influence the rate of embryo growth.
- Embryo growth inhibitors in the pericarp can be overcome either by warm stratification in a substrate or by washing the fruits in running tap water for 48 h. Washing reduces the warm pretreatment requirement by about 6 weeks.
- The optimum temperature of the warm phase is 15C.
- The optimum duration of the warm phase is 16 weeks.
- The duration of the cold phase had a clear effect on the mean germination time. The cold phase should last at least 16 weeks.
- The moisture content of the fruits during the warm phase should be 45% to 55%, during the cold phase 45% (to prevent premature germination).
- Drying after harvest or after stratification gives only small reductions in germination capacity.

WILD CHERRY (*Prunus avium* L.)

Wild cherry seeds need both warm and cold stratification.

Optimal Seed Collection and Handling. Fruits of wild cherry were harvested in three successional years at weekly intervals from 6 weeks before full maturity up to full maturity. Dry weight, desiccation tolerance, and the level of dormancy (measured by mean germination time) of the seeds increased during maturation while moisture content decreased (Jensen, 1996). Sugars (glucose, fructose, and sucrose) increased during maturation (Nowag et al., 1997). All these results indicate that optimal seed quality is provided at full maturity. The maturation phase is delayed by cold springs and summers and hastened by warm springs and summers (Jensen, 1996).

Wild cherry seeds are able to germinate from about 5 to 6 weeks before full maturity, but only with about 40% germination and survival. Fully matured seeds may reach a germination capacity of more than 80%. Because the seeds acquire the ability to germinate late in their development, and since fully mature seeds produce the most vigorous seedlings, premature harvest cannot be recommended (Jensen, 1996).

Pretreatment Conditions. Wild cherry seeds, harvested from 5 different provenances in 1992, 1994, and 1995, were stratified at -5C at a moisture content of approximately 10%, after 2 months or 1, 2, or 3 years storage. Five different stratification treatments were also compared:

1) 2 weeks at 20C, 2 weeks 3C, 2 weeks 20C, 12 to 16 weeks 3C (until germination).

2) 2 weeks at 20C, 6 weeks at 3 to 5C, 2 weeks at 20C, 2 weeks at 3 to 5C, 2 weeks at 20C, and 12 to 16 weeks at 3 to 5C (until germination).
3) Same as Treatment 1 but with an addition of a compost activator from start of stratification.
4) Same as Treatment 2 but with an addition of a compost activator from start of stratification.
5) Same as Treatment 2 but with an addition of a compost activator when 50% of the stones had cracked.

Treatment 2 resulted in the largest number of seedlings in each case — between 2500 and 3000 seedlings per kg (at 30% moisture content) of seeds. It is important that the last cold period is sufficiently long, at least 12 weeks, to reduce the risk of inducing secondary dormancy at temperatures of 20C or more, which easily can happen at spring-time sowing (Nowag et al., 1997).

Currently it is not possible to determine the quantitative requirement for cold stratification. One investigation therefore looked to see if changes in fat, glucose, fructose, sucrose, and starch content could be related to dormancy release and used as markers for evaluating the release of dormancy. However, no correlation between the changes in reserve compound contents and the release of dormancy could be found. (Nowag et al., 1997).

The moisture content of the seeds during stratification should be about three percentage points below the fully hydrated moisture content (fresh weight basis). The full hydration level has to be determined for each seed lot each year, but usually lies around 38%. By aiming for a moisture content of some 35%, dormancy is released effectively, cracking of stones is avoided, and no premature germination occurs (Jensen, 1997).

The testa plays an important role in controlling the elongation and growth of the radicle during warm and cold stratification. In dormant seeds without testa the radicle will start to grow immediately when the temperature reaches 20C. At 4C radicle growth is delayed and will begin after about 20 weeks. In seeds with testa (but without exocarp) radicle growth is restricted at 20C and when such seeds are stratified at 4C they behave in the same way as whole stones (Nowag et al., 1997).

Drying generally leads to cracking of stones in a proportion of the seed lot. The faster the drying and the lower the final moisture content, the larger the proportion of cracked stones. Slow drying at low temperatures (4C) and high relative humidities (30% RH) showed a tendency to reduce germination compared to faster drying at lower RH (Jensen, 1996). Wild cherry seeds should be dried at temperatures between 15 and 20C with a RH of 20%.

Storage of dry (moisture content of 8% to 10%) nonstratified seeds at -5C is possible for up to 3 years without loss of viability and germinability (Nowag et al., 1997).

Redrying and Storage of Pretreated Seeds. After a shortened stratification under the conditions outlined above wild cherry seeds can be dried to a moisture content of about 12% (fresh weight basis) and be stored at 3C for 8 weeks, resulting in only slightly reduced germination capacity. The temperature for storage of pretreated seeds should not be below -3C because large decreases in germination have been observed in seeds stored below this temperature. However if dormancy

is released at higher moisture contents (i.e., fully hydrated rather than at 3 percentage points below full hydration) germination capacity is reduced significantly after drying. Drying the seeds reduces the need for cold treatment, but also induces sensitivity to low germination temperatures (Jensen, 1997).

Conclusions.

- Optimal seed quality is provided at full maturity when germination capacity is 80% or more.
- Optimum stratification is 2 weeks +20C, 6 weeks +5C, 2 weeks +20C, 2 weeks +5C, 2 weeks +20C, and 12 to 16 weeks +5C.
- Optimum moisture content of the stone during stratification is three percentage points below full hydration.
- The seeds should be dried at 15 to 20C and 20% RH.
- Nonstratified seeds with a moisture content of 8% to 10% can be stored at -5C for at least 3 years without loss of germinability.
- Storage of pretreated seeds has not been successful.

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