*Ann. Sci. For.* (1989) 46 suppl., 72s-84s
*Forest Tree Physiology*, E. Dreyer *et al.*, eds.


# Physiological aspects of seed conservation

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## Introduction

Seeds of most woody-plant species are dormant when fully ripe; germination and seedling formation assumes breaking of this dormancy. Preservation of seed viability by proper conservation generally does not contribute to dormancy breaking. After storage, seeds can be as dormant as they were before storage was started. However, whether dormancy is broken before, during or after storage – breaking of dormancy and conservation of seeds should not be regarded separately. Seedling production is not possible from dormant seeds if both dormancy and the tendency to lose viability are not dealt with by proper treatments. We cannot discuss conservation of seeds leaving their dormancy aside. Storage of non-dormant seeds is, except for 'recalcitrant' seeds, much easier.

## Recent trends in seed physiology

In the last few decades, intensive work has been conducted to understand better some basic facts and processes occurring during the development of seeds when they are still on the mother plant and later, after separation from it, in the period between dissemination and formation of seedlings. This period is characterized by breaking of the developmental processes causing dormancy, followed by overcoming this dormancy under conditions making the latter possible. This happens both when dormancy alone is imposed and when it is a very deep dormancy making immediate germination and further growth of the seedling impossible. The reasons for these phenomena can differ. They are mostly multifactorial. Investigators concerned with these problems try to separate the action and effects of individual factors or groups of factors, to exclude in this way interactions with other processes. This is perhaps the only way to conduct such studies but it is obvious that in reality such distinctions do not exist.

The main trends in seed physiology research are reflected by a concentration of studies on the topics enumerated below[1].

1. Role of seed dehydration in the switch from the developmental program of

[1] I would like to express my thanks to Prof. S. Lewak, Institute of Botany, University of Warsaw, for his kind enumeration of recent trends in seed physiology (items 1–5), and for his comments concerning the results obtained to date.

gene expression to the germination program. These studies on the molecular level include identification of specific proteins synthesized during the operation of both programs, among them synthesis of amylolytic enzymes and (important for the understanding of ageing) studies on the mechanism of lesions and repair of DNA. Results obtained so far do not contribute to a better understanding of dormancy (Osborne, 1981; Daussant *et al.*, 1983; Symons *et al.*, 1983; Kermode and Bewley, 1985; Cornford *et al.*, 1987).

2. Hormonal control of metabolic activity during the onset of and release from dormancy. So far, the roles of ABA (abscisic acid) and of gibberellins have been recognized in the initiation and cessation of growth, weakening of endosperm and mobilization of reserves, or in counteracting these processes (Webb *et al.*, 1973a, b; Karssen *et al.*, 1983; Symons *et al.*, 1984; Schopfer and Plachy, 1985). The proposal to call abscisic acid 'dormin' shortly after recognizing its importance for the hormonal control of some processes in growing plants and in seeds reflects best the excessive hopes connected earlier with these studies.

3. Metabolic changes while overcoming dormancy and initiating germination, mainly mobilization of reserves and energy metabolism. Results of studies on the role of an alternative (CN-resistant) electron transport pathway are still controversial. The involvement of the oxidative pentose phosphate pathway in the control of dormancy is evident, but still far from being completely understood (Lewak *et al.*, 1975; Lewak and Rudnicki, 1977; Esashi *et al.*, 1979; Roos, 1980; Pradet, 1982; Köhler and Hecker, 1985).

4. Correlations between various seed organs. Important interactions were detected between various seed structures in their protective (inhibitory), nutritional (nutrient supply) and regulatory roles (Wyzińska and Lewak, 1978; Côme, 1980/1981; Bulard and Le Page-Degivry, 1986; Halińska *et al.*, 1987).

5. Attempts to understand the mode of action of environmental factors, such as light, temperature and moisture. Cellular membranes are being postulated as the sites of primary responses, even a kind of 'memory' is postulated in the membranes (Bartley and Frankland, 1985; Probert *et al.*, 1985a, b; Marbach and Mayer, 1985; Hilton, 1987).

The studies mentioned above were conducted on seeds of annual plants (cereals, leguminous plants, herbaceous perennials). Such investigations on seeds of woody plants, mostly single species or even cultivars from a few genera, concern: apple cultivars *Golden Delicious* and *Antonovka* (Durand *et al.*, 1975; Tissaoui and Côme, 1975; Isaia and Bulard, 1978; Lewak, 1984; Bulard, 1985; Halińska *et al.*, 1987), *Acer* (Nikolaeva, 1967; Szczotka and Tomaszewska, 1979; Pinfield and Dungey, 1985; Szczotka, 1988), *Corylus* (Bradbeer, 1968, 1988), *Fraxinus* (Sondheimer and Galson, 1966) and some other species.

The majority of woody-plant species from the temperate zone produces dormant seeds. Dormancy of seeds of some species *(Pinus sylvestris, Betula alba)* was recognized very late because it is overcome by a very short period of action of red light or a light including the red band of the spectrum (Nyman, 1963; Junttila, 1976).

Some species, among them such important ones as beech and oaks native to Europe, in addition to some coniferous species, produce viable seeds at long time intervals, sometimes every 5–10 yr and these intervals seem to increase as a consequence of air pollution, even in the case of species such as *Picea abies,* which produces cones more frequently (Chałupka and Giertych, 1973). Despite the importance of proper solutions for practical plant production, investigations on the effective and genetically harmless treatments for breaking dormancy, including storage, especially long-term storage, are conducted by only a few research groups concentrating their efforts on these problems (Tyszkiewicz, 1949; Rohmeder, 1951, 1953; Holmes and Buszewicz, 1956; Schönborn, 1964; Buszewicz, 1967; Vlase, 1969; Machaníček, 1973; Suszka, 1974; Bonnet-Masimbert and Muller, 1975, 1977; Bonner, 1978; Suszka and Tylkowski, 1980; Suszka and Kluczyńska, 1980; Muller and Bonnet-Masimbert, 1985; Muller, 1986; Bonner and Vozzo, 1987; Suszka, 1982, 1988; Wang, 1982) and some of them are no longer active.

Of high importance for the elaboration of proper methods of seed handling under controlled conditions in the period between seed collection and seedling emergence are the following: effective methods of estimation of potential germinability of deep-dormant tree-seeds (viability tests developed already in the 20's or 30's (Nelubov, 1925; Piskarev, 1937; Flemion, 1938; Krzeszkiewicz, 1939; Tyszkiewicz, 1939; Lakon, 1950; Lakon and Bulat, 1952; Bulat and Lindenbein, 1961), methods of X-ray testing (Simak, 1957; Simak and Kamra, 1963; Belcher, 1974; Chavagnat, 1984), studies on frost tolerance of seeds in connection with their hydration level (Zakhariev and Tsonev, 1958; Schönborn, 1964) and studies on the mutual interactions between various thermal conditions causing germination or induction of a new dormancy in seeds (Haut, 1938; Crocker and Barton, 1931, 1953; Nikolaeva, 1967, 1977; Suszka, 1976; Edwards, 1986). New phenomena resulting from the pollution of air, soils and waters contribute to a decrease in seed production and further extension of time intervals between seed years. All this makes it more important not only to have short-term storage but especially long-term conservation of seeds of coniferous species and of the much less understood broadleaves, especially shrubs. This is all the more urgent because some ecotypes or even whole species are in danger of disappearing. It is very seldom that seeds of woody-plants are stored in gene banks. Even when they are, this happens often without a well-based knowledge of germination. Only a very few research centers exist where long-term studies on these topics are systematically conducted. Seldom are the even more laborious studies conducted on long-term storage of non-dormant and/or deep-dormant seeds of woody plants, where the whole complex of problems concerning storage, breaking of dormancy, germination and formation of seedlings under controlled conditions, and the latter also in the nursery, is investigated simultaneously.

Special difficulties are connected with investigations on the storage of seeds with a high moisture content, which cannot be dehydrated below a usually high threshold level. The clear separation of all seeds into categories of 'orthodox' and 'recalcitrant' (Roberts, 1975) proved very helpful here. Results of the abovementioned studies are of high interest, not only for practical nursery producers but also for seed banks and other institutions interested in seed reserves. This is all the more important because it has become evident that some species of woody plants are endangered by total extinction either as a consequence of diseases (*Ulmus*) or merciless utilization in earlier times (*Taxus*) or by the most recent consequences of air pollution (*Abies alba* in Central Europe). The recent and rapidly spreading and so feared forest decline ('Waldsterben') concerns not only coniferous species, such as pine, spruce and larch, but also beech and oak forests weakened in East and Central Europe in the early 80's by drought, frost and insect invasions, and attacked finally by plant diseases caused by fungi and viruses.

So far, stratification in a moist medium has been the most promising method of breaking dormancy of woody-plant seeds. Low temperature above the freezing point in autumn, winter and early spring and the elevated temperature in summer in the soil or under more artificial conditions assured a more or less effective preparation of seeds for germination and the emergence of seedlings, whether they needed a cold period only or a cold one preceded additionally by a warm one. When dormancy of seeds has to be broken under controlled conditions, the practical experience collected through centuries by nurserymen and gardeners is practically less useful, because it is based mostly on early, sometimes 'green' collected and therefore non-dried seeds, sown very often even before winter, whether they were subjected earlier to warm stratification under natural conditions (*e.g., Fraxinus excelsior*) or not (*e.g., Acer platanoides*). These modes of pretreating cannot be applied to stored seeds. Seeds of woody plants intended to storage must be completely ripe at collection time and traditional treatments assuring often good results for 'green' collected seeds are ineffective for late collected ripe seeds, which must furthermore be dried for storage and dry-stored. For pretreating seeds under controlled conditions, new methods have to be developed, for each species separately, because even seeds of various species belonging to the same genus can differ substantially in their biological characteristics and their physiological condition. These differences decide the choice of the most useful method of presowing treatment. This can be illustrated with the example of two common genera – *Fraxinus* and *Acer*.

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|---|---|
| *Fraxinus excelsior* | undergrown and dormant embryos, 'orthodox' seeds |
| *Fraxinus ornus* | well-developed non-dormant embryos, 'orthodox' seeds |
| *Acer platanoides* | deep-dormant 'orthodox' seeds |
| *Acer pseudoplatanus* | deep-dormant 'recalcitrant' seeds |
| *Acer saccharinum* | non-dormant 'recalcitrant' seeds |

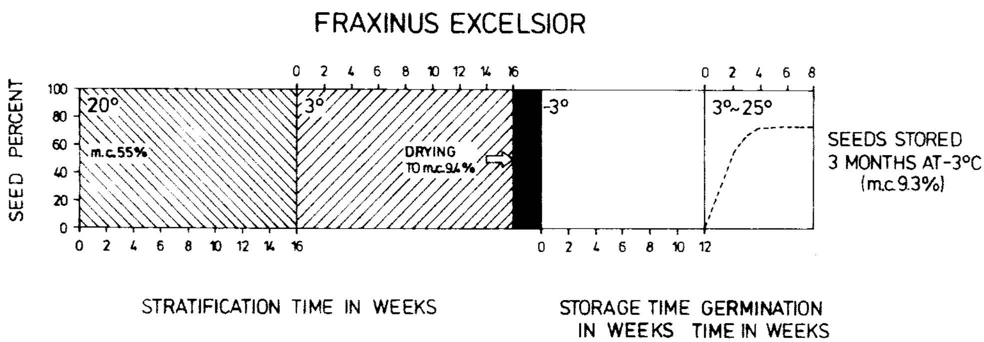


**Fig. 1.** Course of germination of previously dormant *F. excelsior* L. (common ash) 'orthodox' seeds. Freshly collected samaras were dried to a moisture content of 9.3% and were stored at –3°C for 3 mo. After that, they were allowed to imbibe up to a moisture content of 55% and were pretreated by warm stratification at 20°C (16 wk) followed by chilling at 3°C (16 wk), both phases without stratification medium (naked stratification). The pretreated seeds were then dried to an m.c. of 9.4% and were stored again at –3°C in a sealed container for 3 mo. After that, the seeds were placed for germination in a moist medium for 8 wk at an alternating daily temperature of 3~25°C (16 + 8 h/cycle) (T. Tylkowski, by courtesy).

## Promising research directions

*Presowing treatment of dormant 'orthodox' seeds without any medium (naked stratification) (Fig. 1)*

Because of the large volume of the seed/stratification medium mixture, new methods are being developed now based on the already known method of stratification without any medium, the so called 'naked stratification' of imbibed seeds, applied mostly for chilling of seeds of conifers. Our method of stratification without medium resembles that of 'priming' non-dormant vegetable seeds (Heydecker, 1973/1974) with osmotically active solutions of PEG (polyethylene glycol), assuring the retention of a constant level of moisture content in the treated seeds. The latter method has been applied (Simak, 1976) to 'priming' of non-dormant seeds of Scots pine. For beechnuts, we have developed a method based on the same principle and it could be called 'priming' without PEG. Seeds placed in the moist stratification medium imbibe as much moisture as can be taken at the given temperature and the physiological state of the seeds themselves. When working without the stratification medium, the seeds should be brought to and afterwards kept at a level of moisture content high enough for their proper after-ripening, but simultaneously so low that it inhibits germination until the content is increased to a level of hydration permitting germination and seedling formation. In this way, the volume necessary for stratification of seeds mixed with the stratification medium (1:3, v/v) can be reduced to 1/4, *i.e.*, to the volume of the seeds only. The possibility of applying this method to other species of woody-plants is now being studied intensively in the seed laboratories in Nancy–Champenoux and in Kórnik, as is its application to seeds of species demanding not only a cold but also a warm-followed-by-cold treatment. A satisfactory solution to this problem would permit either a reduction of the dimensions of stratification chambers and as a consequence a serious reduction in the cost of their construction and maintenance for at least some important tree and shrub species, or else filling the unchanged chambers with 4 times more seed material.

*Conservation of dormant or non-dormant 'orthodox' seeds (Figs. 2 and 3)*

The 'classic' way of storing seeds characterized by deep dormancy when ripe in the case of the 'orthodox' ones depends upon drying the seeds to a low level and in placing them for shorter or longer periods in sealed non-corrosive and chemically neutral containers at a low temperature. Seeds of temperate zone species should be stored at a temperature just above the freezing point or, even better, below it. For long-term storage, temperature in the range between –5 and –20°C should be preferred, for short-term storage a temperature of –3 to –5°C is sufficient. After storage, the seeds should be defrosted and prepared for germination by a stratification treatment, depending upon the species. When the stratified seeds start to germinate, they should be sown in the nursery or in plastic tunnels or tents. In order to have the termination of stratification and the sowing date coincide, it is necessary to know the necessary period of pretreating the seeds either by a cold only stratification or by a more complex warm-followed-by-cold treatment. Because there is no possibility to adapt methods of pretreating seeds of one species to seeds of another one, even when it belongs to the same genus, seed physiologists must

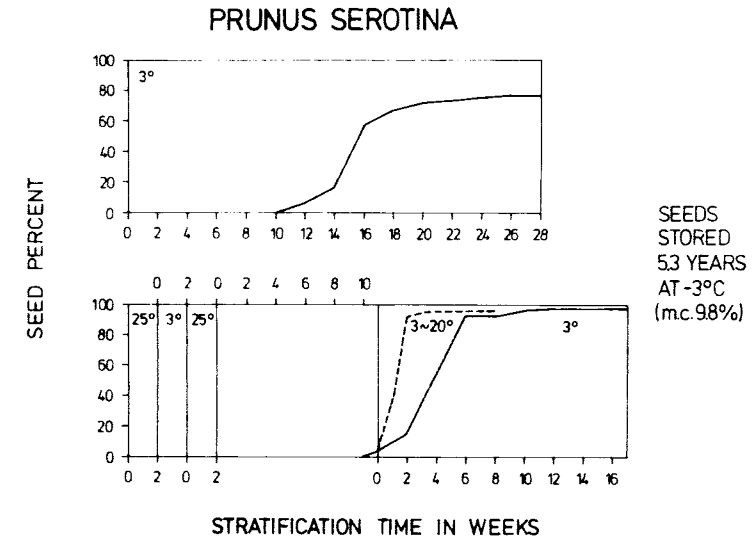


**Fig. 2.** Course of germination of *P. serotina* Ehrh. (black cherry) 'orthodox' seeds. Seeds in intact stones were stored for 5.3 yr at –3°C with a moisture content of 9.8%. After storage, seeds were stratified either only at 3°C or at first for 2 consecutive dormancy-inducing 2 wk treatments at 25°C, separated by a 2 wk break at 3°C, followed by 11 wk at 3°C, *i.e.*, until seeds started to germinate. After that the seeds from this 2nd series were placed for germination at 3°C or at an alternating daily temperature of 3~20°C (16 + 8 h/cycle). All phases were conducted under stratification conditions, *i.e.*, in a moist medium.

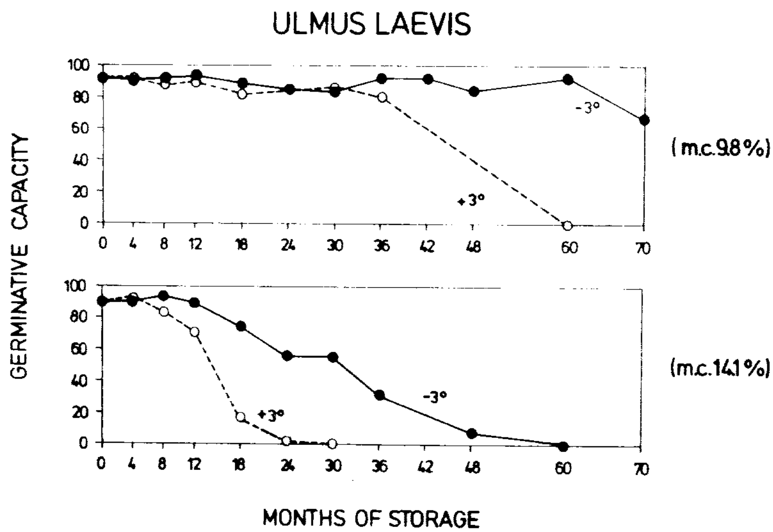


**Fig. 3.** Final levels of germinative capacity of 'orthodox', non-dormant *U. laevis* Pall. (Russian elm) seeds. Fresh seeds were dried to a moisture content of 9.8 or 14.1% and were stored in sealed containers at 3 and –3°C for up to 70 wk. Germination tests at 23°C were repeated after the indicated time intervals (T. Tylkowski, by courtesy).

study the demands of seeds of each species separately. In the case of species with a large area of natural distribution, this has to be done even for single ecotypes from certain regions or from different elevations. Data concerning storage, especially long-term storage, of dormant 'orthodox' seeds of many broadleaf and coniferous woody-plant species are still lacking and intensive investigations are necessary.

*Conservation of dormant and non-dormant 'recalcitrant' seeds (Fig. 4)*

To this category belong completely non-dormant seeds like those of *Quercus robur* or *Acer saccharinum,* semidormant seeds of *Quercus rubra* but also deep-dormant seeds of *Acer pseudoplatanus.* Even 'orthodox' seeds of *Fagus sylvatica* or *Abies alba* and of some other species were earlier regarded as belonging to 'recalcitrant' seeds. 'Orthodox' seeds are difficult to store because their high moisture content has to be retained to assure seed viability. For each species, individual methods of storage have to be elaborated. Great difficulties are created in the case of seeds of 'recalcitrant' species by their often enormously high respiratory activity resulting from the high hydration level of the living tissues. This activity can easily cause overheating of seeds when stored under conditions insulating them from the cooler outer air. The high moisture content is also responsible for the low frost resistance of such seeds, making utilization of low subfreezing temperature below –3°C impossible. Thanks to the efforts of a few investigators (Holmes and Buszewicz, 1956; Messer, 1960; Suszka and Tylkowski, 1980, 1982; Muller and Bonnet-Masimbert, 1984; Bonner and Vozzo, 1987), solutions exist already permitting storage of 'recalcitrant' seeds of at least some species over 2–3 seasons. However, these methods should be improved and elaborated for species still not investigated.

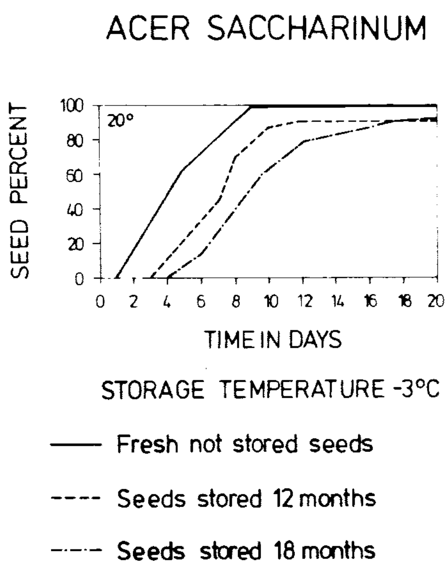


**Fig. 4.** Course of germination at 20°C of 'recalcitrant', non-dormant *A. saccharinum* L. (silver maple) seeds. Germination tests were started immediately after collection of fresh seeds or after storage at –3°C for 12 or 18 mo. The initial moisture content of the intact samaras was 51.8%, that of the enclosed seeds 60.0% (T. Tylkowski, 1985).

*Conservation of already pretreated seeds (Figs. 1 and 5)*

In the last few years, attempts have been made to reverse the sequence of operations connected with storage and breaking of dormancy of deep-dormant seeds. This could be achieved by pretreating them first, shortly after collection, without allowing them to lose the high initial viability and high moisture content, and to store them afterwards at low temperature. There are two ways to do this after dormancy has been broken. The first way can

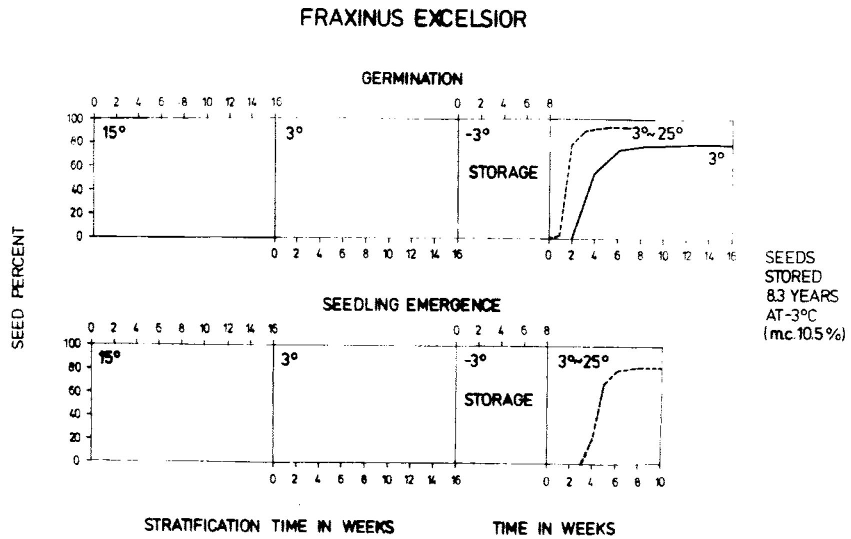


**Fig. 5.** Course of germination and of seedling emergence of previously dormant, 'orthodox' *F. excelsior* L. (common ash) seeds. Seeds (in intact samaras) were dried to a moisture content of 10.5% and were stored for 8.3 yr at –3°C in a sealed container. After storage, the seeds were stratified at first at 15°C for 16 wk followed by 16 wk at 3°C. After that, the stratification medium containing the seeds was frozen at –3°C for 8 wk. After defrosting, germination tests were started at 3° or at 3~20°C (16 + 8 h/cycle) in addition to a seedling emergence test at the latter alternating temperature.

be realized by drying the already non-dormant, highly imbibed seeds to a low moisture content level, followed by dry and cold storage in sealed containers (like the 'classic' way). The other solution is to freeze the moist stratification medium with the already pretreated seeds and to sow the seeds or the seed/medium mixture after a period of cold storage followed by defrosting of the medium. Some data are available from the 60's and 70's (Vanesse, 1967; Hedderwick, 1968; Barnett, 1972) on drying of already stratified, previously dormant *Pinus ponderosa* and *Pseudotsuga menziesii* seeds, and data from the early 50's (Rohmeder, 1951) on drying of artificially highly hydrated non-dormant *Pinus sylvestris* and *Picea abies* seeds. Other data, from the early 30's up to the early 80's, concern dormant seeds of conifers (Edwards, 1982, 1986) or of fruit tree species from the subfamilies Pomoidae and Prunoidae of the Rosaceae family (Crocker and Barton, 1931; Haut, 1932; Stepanov, 1955; Visser, 1956; Decourtye and Brian, 1967; Kaminski and Rom, 1973). These data are contradictory but indicate for seeds of some species that seed populations with at least some seeds already after-ripened can be dried without serious decrease of their viability and without an induction of secondary dormancy. The contradiction lies in that some authors believe that seeds treated in this manner should be restratified, while other authors deny this necessity. This problem has been thoroughly studied by us (Grzeskowiak and Suszka, 1983) in the model of *Prunus avium* seeds in intact stones and it was found that the most favorable date of sowing coincides with the start of germination. The already stratified stones when dried to 10% moisture content can be stored up to 1 yr but germinability decreases to half of its initial level. Positive results were obtained in our studies for beechnuts (Suszka, 1975). Beechnuts can be dried after chilling without medium at a controlled hydration level, extended until all germinable seeds are ready to germinate but still do not germinate. When dried in a forced air stream at a moderate temperature they can be stored like other 'orthodox' seeds, *e.g.*, at –10°C for 100 days without any decrease of germinability. Our attempts to extend the storage period up to 1 yr did not assure positive effects, however, before treatment, the seeds had already been dry-stored at a low temperature for more than 1 yr. When fresh seeds were used for such a treatment, germinability remained high even after nearly 6 yr of storage (Muller and Bonnet-Masimbert, 1982). These authors also report promising results for seeds of *Fraxinus excelsior* and *Prunus avium*. When these first results will be confirmed by studies on broader seed material, commercial storage of already pretreated seeds deprived of their dormancy can perhaps become reality.

Positive solutions to conservation of already pretreated seeds would (at least for some species) in properly equipped stations or facilities enable seedmen or seed trade companies to deliver to nurseries seeds ready to germinate after sowing. Which species could be adapted to this seed strategy must be thoroughly studied.

Introduction of new methods of seed handling necessitates the building of new facilities or an adaptation of the old ones. In such facilities, well-trained personnel, backed by a laboratory providing the necessary data, would be able to treat each seed lot separately, according to the individual life history of each lot and thus determine individual differences between seed lots. Other sources of differences, even between seed lots of the same species are connected with provenances, seed years, characteristics of individual trees from the same stand or even the conditions of pollination.

## Acknowledgments

The data presented in the figures originate exclusively from investigations conducted in the Institute of Dendrology in Kórnik.

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