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Conditions for the breaking of dormancy and germination of the seeds of *Aesculus hippocastanum* L.

INTRODUCTION

The dormancy of horse chestnut seeds has been little studied so far. The few informations that can be found in the literature deal with instructions for storing and presowing treatment of the seed which are all based on casual observations rather than on systematic studies. The paper given here deals with a study conducted in the years 1963/65, which attempted to fill the gap in our knowledge on the physiology of horse chestnut seed dormancy.

Tyszkiewicz (7) and Krüssmann (4) suggest in agreement with nursery practice to sow the nuts in the autumn immediately after collection, or else to dry them slightly and store in moist sand in a pit until sowing time in the spring. A storage of the seeds loose in sacks at a temperature 4,5–10°C. caused a reduction of the seed viability over a period of 5 months from 90% to 29% (reported by Holmes and Buszewicz (3) after the Woody-Plant Seed Manual (1)). These authors consider chestnuts as a group of species which have seeds, that on reaching full ripeness are characterized by high moisture content and quick loss of viability under conditions of desiccation or otherwise unfavourable for germination.

The seeds of chestnuts are characterized by a dormancy of the embryo which according to the authors of the Woody-Plant Seed Manual can be overcome by a stratification of about 120 days in moist sand at a temperature of about 5°C. This observation refers to the following species: *Aesculus hippocastanum* L., *A. glabra* Willd. and *A. octandra* Marsh. The seeds of *A. californica* Nutt. do not require stratification, and in nature they usually germinate already in November after the onset of winter rains. However the authors of these observations point out that as yet the optimal stratification periods for the seeds of different species have not been established yet. All that is known is that a 60 day stratification of the seeds of *A. hippocastanum* and *A. glabra*, and a 90 day stratification of the seeds of *A. octandra* will suffice.

The seeds of chestnuts prepared in the proper fashion will germinate in a high percentage. Sound seeds of *A. hippocastanum* germinate in the spring over a period of 3 weeks in about 90% (Krüssmann 4). Tyszkiewicz (7) recommends that germinability tests of the seeds should be performed in sand at a temperature of 18—20°C for a period of 42 days. This of course, refers to seeds which have been prepared for germination. The authors of the Woody-Plant Seed Manual recommend for germinability tests of unstratified seeds more than 230 days for *A. hippocastanum* and *A. glabra*, more than 160 days for *A. octandra* and only 20 days for *A. californica*. To establish the germinability of stratified seeds of *A.* octandra a test of 20 days suffices. The temperature recommended for these tests alternating in a daily cycle from 20—30°C is considered by the authors of the Manual as not the optimal temperature for germination of the chestnut seeds.

The external coat of the horse chestnut seeds is covered according to Côme (2) by a layer of wax, which prevents the access of atmospheric oxygen to the embryo. The gas exchange can take place freely only through the porous hilum (a dull, light brown area on the surface of the seed, which forms in the place of the original attachment to the funiculus). Krüssmann (4) recommends that the seeds be sown with the hilum facing down. Since the seed germination is hypogeous such positioning of the seed will permit a proper development of the root and the epicotyl.

Holmes and Buszewicz (3) believe that the best conditions for a short term storage of the horse chestnut seeds are provided by a cool moist environment. In the Woody-Plant Seed Manual (1) it was already suggested that the storage of these seeds in closed containers at a temperature of 0°C or slightly lower can be useful for the preservation or extension of their viability. The isolation of the seeds from the outside atmosphere prevents their desiccation. May (5) stored fresh seeds of *A. hippocastanum* and *A. glabra* in polyethylene sacks for a period of 175 days at temperatures of —0.5°, 4.4° and 7.2°C.

The lowest temperature —0.5°C, proved most advantageous, since the percentage of decayed seeds was lowest and the percentage of germinating seeds and surviving one year old seedlings was highest. In another experiment May stored the seeds of both these species in the same manner at a temperature of —0.5°C over a period of 130 days. In this case the percentage of decayed seeds was minute (0.1%) after the end of the storage period. The seeds germinated within a few days from sowing and the percentage of surviving seedlings was here also very high. The most important find of May is the establishment of the fact that after storage of the seeds in polyethylene sacks at a temperature close to 0°C for a period of 130 or 175 days the seeds germinated immediately.

In the present study it was attempted to establish the relation between the degree of dormancy of the seeds of *A. hippocastanum* L. and temperature. For the studies fresh seeds and seeds with a reduced water content were used for stratification in early autumn or for storage over the winter in closed containers and sowing in the spring.

METHODS

For the experiments a mixture of seeds from several trees was used. The seeds were collected after fruit dehiscence in 1963 and 1964 from *A. hippocastanum* trees growing in the Kórnik Arboretum near Poznań.

From the first to the last day of collection, that is over a period of 10 days the seeds were stored in a shaded, cool and moist place. Seed moisture was lowered by spreading the seeds as a thin layer in a shaded location, not too drafty and maintained at room temperature.

The seed moisture was measured by drying the cut up material at 105° C, for a period of 48 hours. The moisture content is presented in this paper as a percentage of fresh weight.

When testing the viability of the seeds the cutting test was used.

The seeds were stored in bottles filled to capacity which in the 1963/64 season were closed with corks and sealed with paraffin and in the 1964/65 they were closed by a double layer of polyethylene 0.1 mm thick which was tightly bound and the ends were fixed with a piece of plaster to the bottle necks.

The stratification was performed in a moist mixture of sand and peat (1 : 1 by volume) and the volume of this mixture exceeded the volume of the seeds twofold. For the stratification plastic boxes with perforated lids were used. All the studies were performed in our phytotron. Fluctuations of temperature in the phytotron chambers did not exceed $\pm 0.5°$ C.

The stratified seed lots were checked depending on the experiment at 5, 7 or 14 day intervals. Simultaneously the following functions were performed: the mixture of seeds and medium were aerated, moisture loss was replaced, the number of radicles and epicotyls that have emerged was counted, and the decayed and fully germinated seeds (with roots and epicotyl) were removed. The seeds were considered as germinated when at least 5 mm of radicle has emerged through the seed coat, and for the epicotyl when stem apex emerged from between the cotyledon stalks.

All the variables in the experiment were replicated 4 times using 50 seeds per replicate. All the numerical values were converted into percentages, and after calculation of the means these were presented graphically in the form of cumulative curves for germination.

In the 1963/64 season the breaking of dormancy and germination of the seeds of *A. hippocastanum* was studied under the following conditions:

a) fresh seeds (49% moisture content) — stratification at temperatures of 1°, 3°, 5°, 10°, 15°, 20° and 25° C, started on the 10th of October 1963,

b) seeds slightly dried (38% moisture content) — stratification at temperatures of 3°, 5°, 10° and 15° C, started on the 26th of October 1963,

c) seeds slightly dried (38% moisture content) — stored for 100 days (from 26.X.1963 to 29.I.1964) in tightly sealed bottles at a temperature of 3° C followed by stratification at temperatures of 3°, 5° and 10° C,

d) seeds slightly dried (38% moisture content) — stored for 190 days (from 26.X.1963 to 21.IV.1964) in tightly sealed bottles at a temperature of 3° C, then stratified at a temperature of 10°, 15° and 20° C. Stratification was started on the 21st of April 1964.

After 20 weeks (for a and b) or 8 weeks (for c and d) of stratification from each experiment two replicates were removed from the experiment and transferred to a uniform temperature of 20°C, while the remaining two replicates were left in the temperature assumed at the onset of the experiment. A schematic representation of the experiments *a* — *d* is given in fig. 1.

During the 1964/65 season the studies were continued, using only fresh seeds with a moisture content of 48,6%. In the first series of experiments a stratification of seeds at a temperature of 5°C was employed lasting in various experiments, established independently of each other, for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 weeks, after which the stratification temperature was raised to 20°C for a period of 8 weeks.

In the second experimental series fresh seeds were stored for 187 days (from 15.X.1964 to 21.IV.1965) in glass containers filled to capacity with seeds, sealed with polyethylene (see above) and placed in temperatures of 1°, 3° and 5°C. On completion of the storage period and having checked the viability of the seeds, some of the seeds were stratified for 8 weeks at temperatures of 10°, 15° and 20°C, while the rest of the seeds were used for field experiments established simultaneously in the form of random blocks with 5 replicates (5×50 seeds). In this last experiment the seeds were sown at a depth of 5 cm with the hilum facing down. The stratification and field sowing played the role of a germination test.

A schematic representation of the 1964/65 experiments is given in fig. 2.

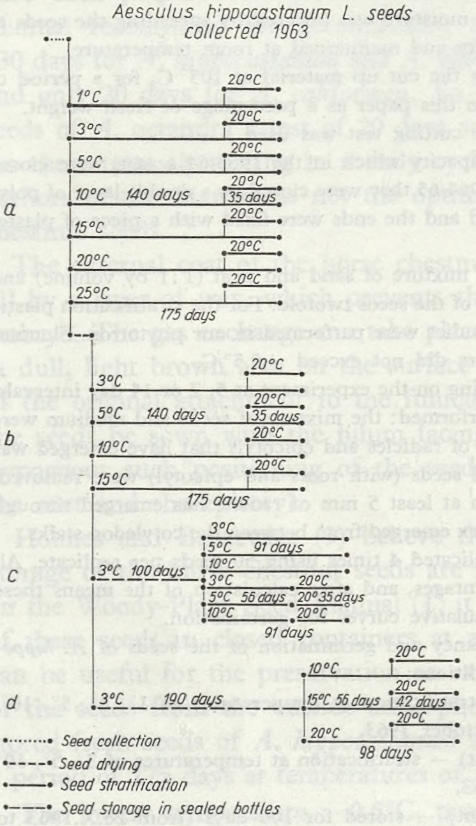

Fig. 1. Pattern of the experiments in 1963/64

a — fresh seeds, moisture content 49%, *b* — partly dried seeds, moisture content 38%, *c* — partly dried seeds, moisture content 38%, stored after drying for 100 days in sealed bottles at 3°C, *d* — partly dried seeds, moisture content 38%, stored after drying for 190 days in sealed containers at 3°C

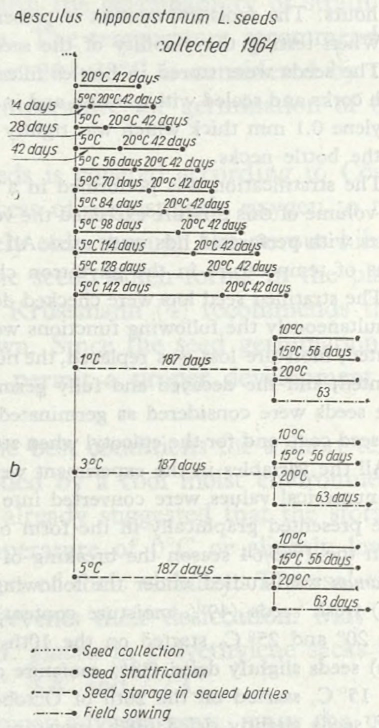

Fig. 2. Pattern of the experiments in 1964/65

a — fresh seeds, moisture content 48,6%, *b* — fresh seeds, moisture content 48,6%, stored after collection for 187 days at 1°, 3° and 5°C in bottles sealed with a double polyethylene sheet

RESULTS

THE BREAKING OF DORMANCY IN FRESH SEEDS

Results of experiments on the stratification of freshly collected seeds of the horse chestnut (49% moisture content) are presented in fig. 3. From this experiment the following conclusions can be drawn. Constant temperatures of 1°, 3°, 5° and to some extent also 10°C, have enabled the seeds to move from the dormant condition to germination. The most effective temperature proved to be 5°C. At temperatures of 15° and 20°C, almost all the seeds remained in the dormant state, however, quite unexpectedly a certain percentage of seeds germinated when stratified at 25°C. The greatest majority of seeds that were

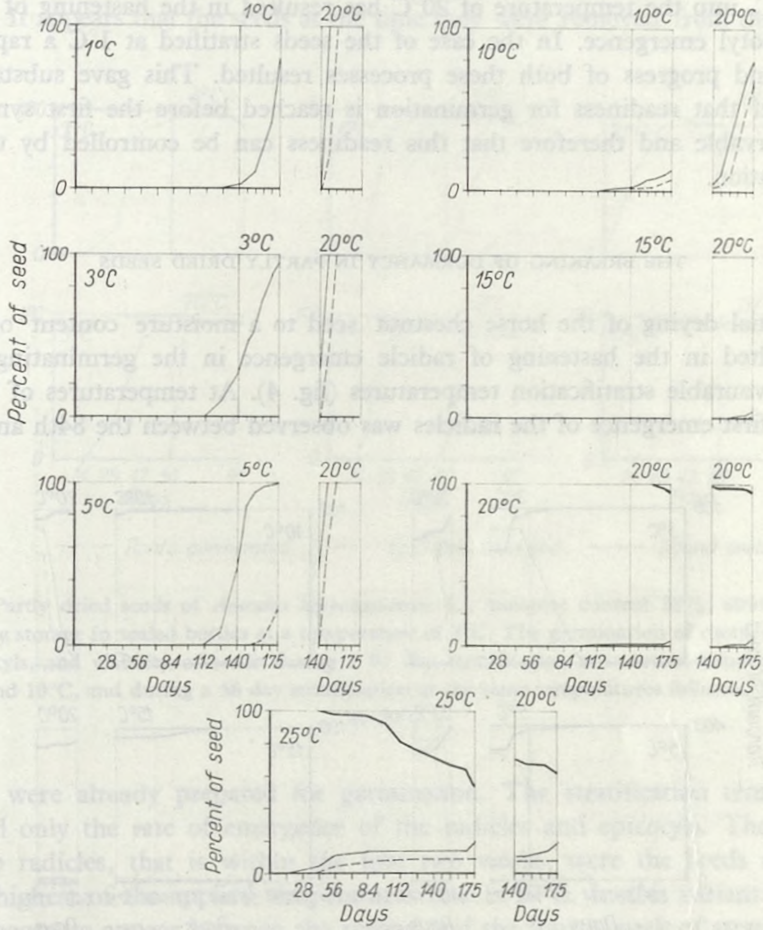

Fig. 3. Fresh seeds of *Aesculus hippocastanum* L., moisture content 49%, stratified immediately after collection. The germination of roots, emergence of epicotyls and the viability of seeds during a 175 day stratification at constant temperature of: 1°, 3°, 5°, 10°, 15°, 20° and 25°C, and during a 140 day stratification at the same temperatures followed by 35 days at 20°C

stratified at this high temperature suffered from decay and died, whereas seeds which were stratified at lower temperatures remained viable. These have maintained throughout the duration of the experiment, that is for 175 days, an almost unchanged viability.

At temperature of 5°C which is most advantageous for the breaking of dormancy in fresh seeds the first germinating seeds were observed between the 112th and 126th day of stratification. At the 25°C stratification the first germinating seeds were already seen between the 14th and 28th day.

The transfer of seeds stratified for 140 days at temperatures in the range 1°—10°C, into the temperature of 20°C has resulted in the hastening of radicle and epicotyl emergence. In the case of the seeds stratified at 1°C a rapid initiation and progress of both these processes resulted. This gave substance to the belief that readiness for germination is reached before the first symptoms are observable and therefore that this readiness can be controlled by thermic manipulation.

THE BREAKING OF DORMANCY IN PARTLY DRIED SEEDS

The partial drying of the horse chestnut seed to a moisture content of 38% has resulted in the hastening of radicle emergence in the germinating seeds under favourable stratification temperatures (fig. 4). At temperatures of 3° and 5°C the first emergence of the radicles was observed between the 84th and 98th

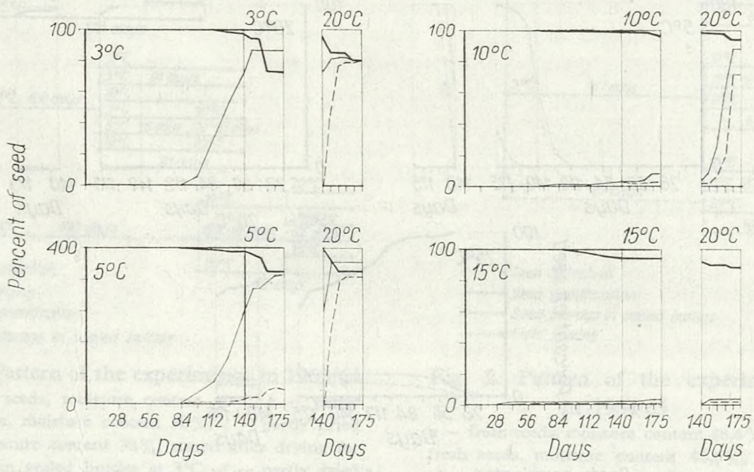

Fig. 4. Partly dried seeds of *Aesculus hippocastanum* L., moisture content 38%, stratified immediately after drying. The germination of roots, emergence of the epicotyls and the viability of seeds during a 175 day stratification at constant temperatures of: 3°, 5°, 10° and 15°C, and during a 140 day stratification at the same temperatures followed by 35 days at 20°C

day of stratification. The emergence of epicolys was noted during the 175 days of stratification only at temperature of 5°C and even here not in many seeds. The change of stratification temperature from 3°, 5° or 10°C to 20°C enabled the germination of the apparantly dormant seeds that were kept under 10°C and permitted the emergence of plumules in all the variants.

THE BREAKING OF DORMANCY IN SEEDS THAT WERE PARTIALLY DRIED AND STORED IN SEALED CONTAINERS

The results of experiments on stratification of partially dried seeds that were stored in tightly sealed containers for 100 days at 3°C are basically different from the results obtained with fresh or partly dried seeds that were not stored (fig. 5). It appears that the seeds at the time they were removed from the storage

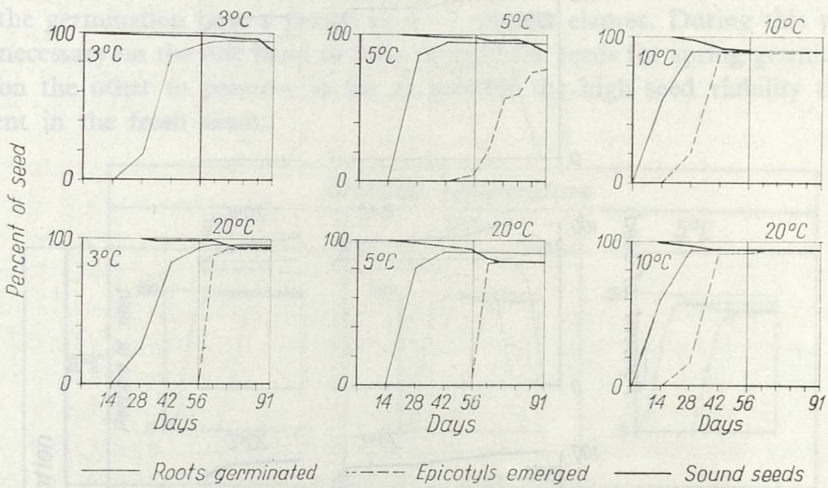

Fig. 5. Partly dried seeds of *Aesculus hippocastanum* L., moisture content 38%, stratified after a 100 day storage in sealed bottles at a temperature of 3°C. The germination of roots, emergence of epicotyls, and viability of seeds during a 91 day stratification at constant temperatures of: 3°, 5°, and 10°C, and during a 56 day stratification at the same temperatures followed by 35 days at 20°C

bottles were already prepared for germination. The stratification temperature affected only the rate of emergence of the radicles and epicotyls. The first to develop radicles, that is within the first two weeks, were the seeds stratified at the highest of the applied temperatures that is 10°C. In this variant the epicotyls began to appear between the second and the fourth week of stratification. In the case of seeds stratified at 3° and 5°C the plumules began to develop rapidly only after the seeds were transferred to 20°C. A maintenance of such seeds at constant low temperature has retarded the emergence of epicotyls (at 5°C) or totally inhibited their growth during the experimental period (at 3°C).

On the basis of the above mentioned experiments it was concluded that a raising above 10°C of the temperature at which the seeds were stratified or sown, following their storage at a lowered temperature, should result in an even greater increase in the germination rate.

This suggestion has found confirmation in the results of the experiments in which the seeds partly dried after collection to a moisture content of 38%, were stored for even longer, namely for 190 days (to the 21st of April 1964), in the sealed bottles at 3°C. In this experiment higher stratification temperatures were used, 10°, 15° and 20°C. Here similarily as in the case of seeds stored for only 100 days the seeds on removing them from the bottles were completely ready for germination, and the stratification temperature only regulated the number of radicles and epicotyl emerged (fig. 6). The first to germinate were

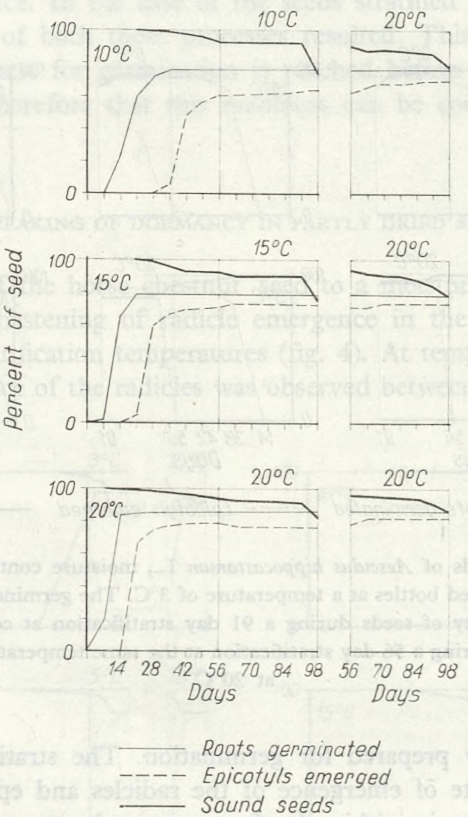

Fig. 6. Partly dried seeds of *Aesculus hippocastanum* L., moisture content 38%, stratified after 190 day storage in sealed bottles at a temperature of 3°C. The germination of roots, emergence of epicotyls and seed viability during a 98 day stratification at a constant temperature of 10°, 15° and 20°C, and during a 56 day stratification at the same temperatures followed by 42 days at 20°C

the seeds stratified at the highest of the tried temperatures, namely 20°C. In this experiment some seeds tended to die off during stratification, and in some variants this affected a substantial portion of the seeds. It is very likely that this was caused not by the storage conditions before stratification, but by an inadequate method of conducting the stratification itself. The seeds were stratified in containers covered by lids with a small number of holes. The difficulity of gaseous exchange for the germinating seeds could have been the limiting factor in maintaining of them in a viable state.

THE BREAKING OF DORMANCY IN FRESH SEEDS STORED IN CONTAINERS CLOSED WITH POLYETHYLENE SHEETS

The stratification of horse chestnut seeds in a fresh or partly dried condition soon after collection in the autumn results in their germination at an unfavourable time, in winter. Thus it is necessary to develop such methods of treating the seeds as to obtain their numerous and rapid germination in the springtime. In the conditions of the temperate zone, between the seed fall in horse chestnut and the germination time a period of 6—7 months elapses. During this period it is necessary on the one hand to fully prepare the seeds for spring germination and on the other to preserve as far as possible the high seed viability that is present in the fresh seeds.

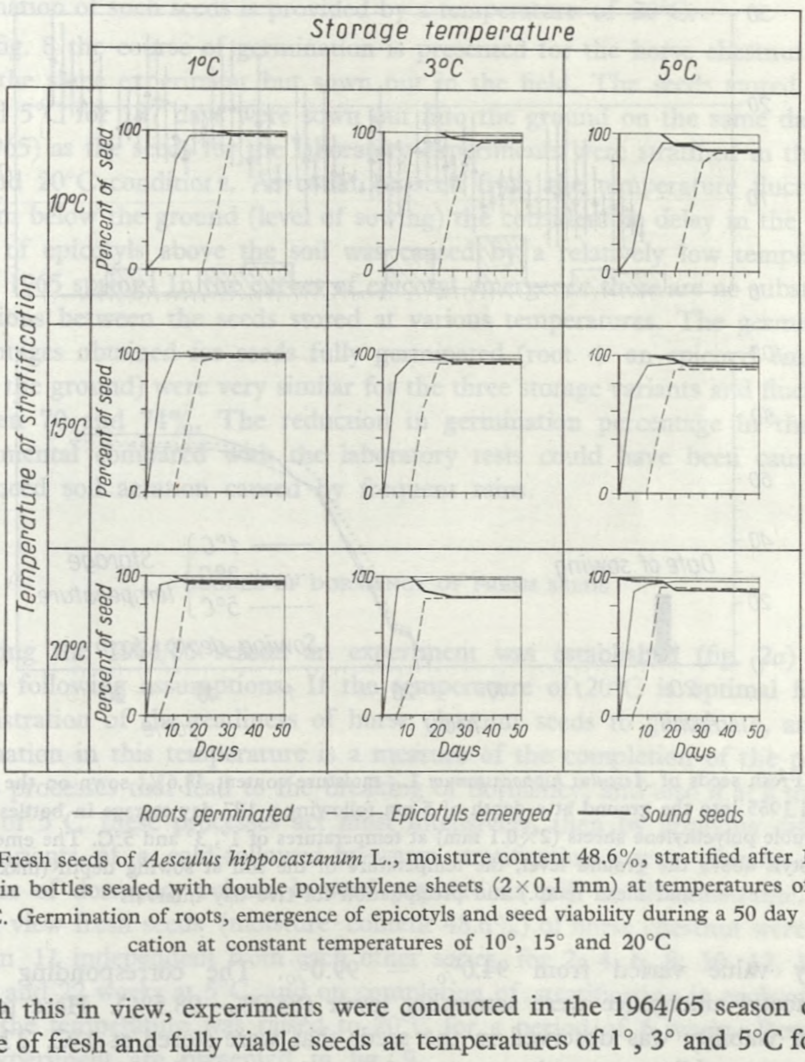

Fig. 7. Fresh seeds of *Aesculus hippocastanum* L., moisture content 48.6%, stratified after 187 day storage in bottles sealed with double polyethylene sheets (2×0.1 mm) at temperatures of 1°, 3° and 5°C. Germination of roots, emergence of epicotyls and seed viability during a 50 day stratification at constant temperatures of 10°, 15° and 20°C

With this in view, experiments were conducted in the 1964/65 season on the storage of fresh and fully viable seeds at temperatures of 1°, 3° and 5°C for 187 days in containers closed with polyethylene sheets. These seeds were tested for germination under conditions of stratification at temperatures of 10°, 15° and 20°C and in a field trial. Results of these experiments are presented in figs 7 and 8. The percentage of healthy seeds at the end of storage was as follows depending on its temperature: 1°C — 96,5%, 3°C — 92,8%, 5°C — 86,1%. About ½ of the dead seeds were covered by a mould. For the germination tests only apparently sound, unmoulded seeds were used. Cutting tests of these selected seeds gave the following level of viability in relation to the storage temperatures: 1°C — 100.0%, 3°C — 92.8%, 5°C — 90.0%. From these results it appears that the 1°C storage temperature gave the lowest losses (3.5%) during the 6 months of storage. After stratification of the seeds all have germinated very quickly and in a large percentage. The germi-

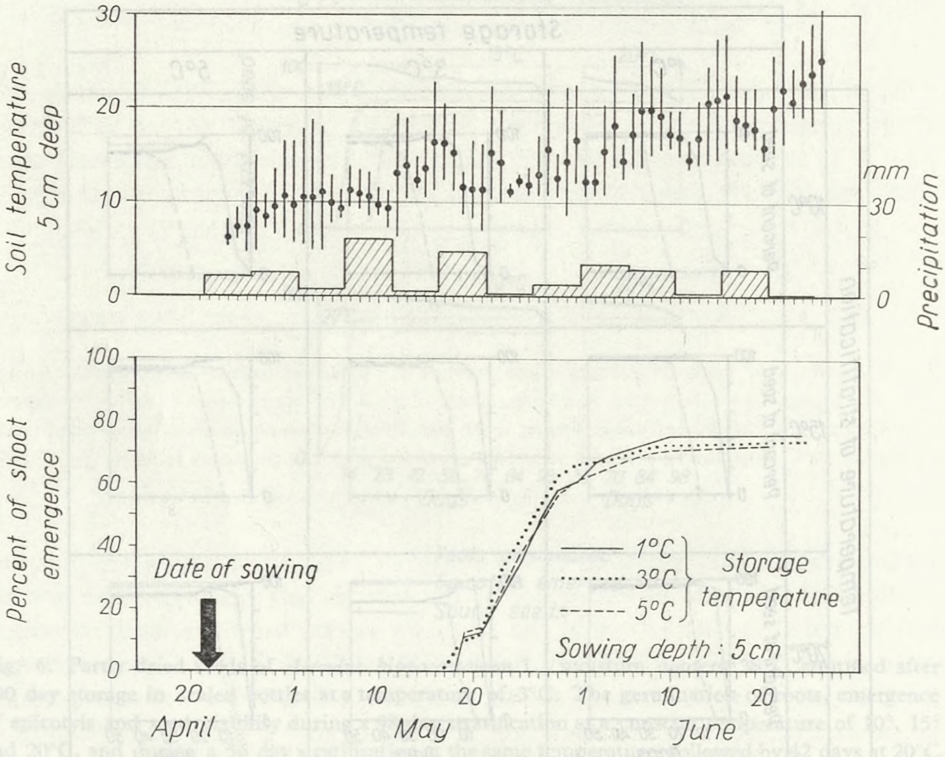

Fig. 8. Fresh seeds of *Aesculus hippocastanum* L., moisture content 48,6%, sown on the 22 nd. of April 1965 into the ground at a depth of 5 cm following a 187 day storage in bottles sealed with double polyethylene sheets (2×0.1 mm) at temperatures of 1°, 3° and 5°C. The emergence of epicotyls above the ground level, the temperature of the soil at sowing depth (max., min. and mean temp.) and precipitation for five day intervals

nability value varied from 94.0% — 99.0%. The corresponding values for epicotyl emergence were somewhat lower (85.0%—98.5%). Here too the highest viability was observed in the seeds that were stored at 1°C. The first and most rapidly to germinate were the seeds stratified at the highest temperature (20°C) and the slowest germination was in the seeds stratified at the lowest temperature (10°C). The temperature of storage prior to stratification did not have an effect on the course of germination. The delay in epicotyl emergence in relation to the time of root germination was significantly a function of the germination temperature. At 10°C it was about 20 days, at 15°C it was 10—12 days and at 20°C it was only 5—10 days.

Summarizing the results presented above it needs to be stated that from all the thermal variants tried, the most satisfactory conditions for the breaking of dormancy and maintenance of high viability are created by a storage in sealed containers at a temperature of 1°C. In these conditions fresh seeds with a high moisture content can be stored in containers closed with polyethylene sheets for a period of 6 months almost completely without losses. The best germination of such seeds is provided by a temperature of 20°C.

In fig. 8 the course of germination is presented for the horse chestnut seeds from the same experiment but sown out in the field. The seeds stored at 1°, 3° and 5°C for 187 days were sown out into the ground on the same day (21. IV. 1965) as the seeds for the laboratory experiments were stratified in the 10°, 15° and 20°C conditions. As could be seen from the temperature fluctuation at 5 cm below the ground (level of sowing) the considerable delay in the emergence of epicotyls above the soil was caused by a relatively low temperature of the 1965 spring. In the curves of epicotyl emergence there are no substancial deviations between the seeds stored at various temperatures. The germination percentages obtained for seeds fully germinated (root + an epicotyl emerging above the ground) were very similar for the three storage variants and fluctuated between 70 and 74%. The reduction in germination percentage in the field experimental compared with the laboratory tests could have been caused by a reduced soil aeration caused by frequent rains.

DEGREE OF DORMANCY OF FRESH SEEDS

During the 1964/65 season an experiment was established (fig. 2*a*) based on the following assumptions. If the temperature of 20°C is optimal for the demonstration of the readiness of horse chestnut seeds to germinate, and the germination in this temperature is a measure of the completion of the physiological processes that lead to the breaking of dormancy, and also if at a temperature of 5°C these processes act most intensively, then by applying different time variants of a cold-followed-by-warm stratification a true picture of the progress of dormancy release in seed populations will be demonstrable. With this in view fresh seeds (moisture content 48.6%) of horse chestnut were stratified in 11 independent from each other series, for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 weeks at 5°C, and on completion of stratification in each of these series the temperature was raised to 20°C for a period of 6 weeks. Results of this experiment are presented in fig. 9.

It was found that in a population of seeds there are individuals reacting very unevenly to the applied temperatures. In a certain though small percentage, seeds germinated even in the control series which was not subjected to a cold stratification. In the remaining variants of the experiment with a gradually increasing period of stratification at 5°C a definite regularity was observed. The longer was the period of chilling preceding the increase of temperature to 20°C the earlier, more rapidly and more numerously did the seeds germinate in the higher temperature. Already the shortest periods of treatment with the 5°C temperature were sufficient to permit a certain percentage, increasing with an increase of the chilling time, of seeds to move from the dormant state to the active growth of root and epicotyl.

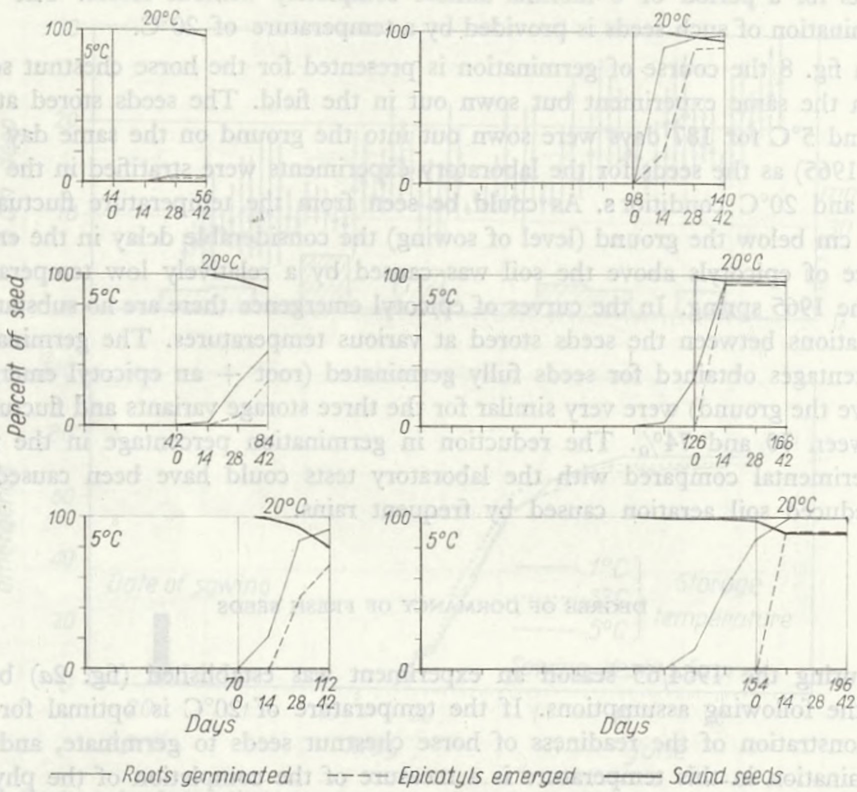

Fig. 9. Fresh seeds of *Aesculus hippocastanum* L., moisture content 48.6%, stratified immediately after collection at a temperature of 5°C for 14, 42, 70, 98, 126 and 154 days followed by a temperature of 20°C for 42 days. The germination of roots, emergence of epicotyls and seed viability during stratification

When extending the period of chilling above 98 days, the onset of germination and its further course was already noticable during the cold stratification. On transfer to 20°C very quickly because within 2 weeks, a germination of all the remaining sound seeds was observed. If the period of chilling exceeded

112 days also the emergence of epicotyls was just as rapid. It appears that if instead of 42 days a longer period of treatment with the 20°C was used, the percentage of seeds with an actively growing root and shoot would have been larger even in the variants with the shorter period of cold stratification. This indicates that in the variants with 28, 42 and 56 days of cold stratification at 5°C the course of germination has not been completed.

On the basis of the results of the experiment described above it can be stated that, individual seeds of horse chestnut react unevenly to the thermal conditions of the environment, even when this environment creates optimal conditions for the breaking of dormancy. The range of reactivity of the seeds within a population appears to be very wide. It would remain to establish whether there is any relation between this range of variability and the origin of seeds from individual trees. The purpose of these studies is not so much an establishment of a uniform period of dormancy under specific conditions, but the experimental delimination of conditions under which it is possible to obtain a most uniform reaction of the seeds to the action of factors favourable to germination. With the purpose of the investigation so defined it is possible to make the following conclusions from the studies conducted so far. The longer is the time period during which the seeds are subjected to the action of low temperature the greater will be the number of seeds ready for rapid germination when the temperature is raised. Thus it would appear advisable to prolong the cold stratification until the maximal number of seeds is ready for germination, but the germination will still be impossible at the stratification temperature. Seeds which are treated so will germinate quickly and in large numbers when the temperature is raised. In our experiment such conditions were ensured by a 98—112 day stratification at a temperature of 5°C. The moment of emergence of the first radicles indicated here the date of sowing of the seeds at 20°C, which provides conditions for energetic germination. In this way an almost uniform reaction of the whole population was obtained whereas the individual seeds have various requirements and reach a readiness for germination at various times.

DISCUSSION

The scope of the studies presented in this paper covers two very distinct groups of problems. One concerns the dormancy and conditions for germination of horse chestnut seeds, stratified immediately or shortly after collection. The second concerns the storage of the seeds and the germination of seeds placed under appropriate conditions on completion of the storage period.

When these experiments were planned, the author was not aware of the studies conducted by May (5), and therefore he expected that the seeds stored during the winter period in closed sealed containers, under low temperature conditions would require the same pre-sowing treatment as the seeds stratified immediately after collection.

Freshly collected seeds, characterized by a high moisture content, reaching up to 50% of fresh weight and dried seeds with a moisture content of 38%, require for the commencement of germination 112—126 and 84—98 days respectively under 5°C or a similar temperature. A certain lowering of the moisture content results in a shortening of the period needed to prepare the seeds for germination by 28 days, that is 1/4 of the time period needed to initiate the germination of undried seeds. This would indicate that there is a relation between the degree of moisture content of the tissues and the reaction of the seeds to temperatures favourable to the breaking of dormancy. This relation was not observed so far in seeds that are sufficiently hydrated, and thus the data available in the literature, meager though they are, have to be considered as not fully trustworthy, where they pertain to length of time needed to prepare the seeds for germination, since they do not provide information about the physiological state of the seeds.

Under optimal thermal conditions of cold stratification, that is at 5°C, the peak time for mass germination in the studied populations of seeds fell on the period between the 126th and 147th day for fresh seeds and between the 98th and 147th day for partly dried seeds. The time of germination for seeds stratified fresh or partly dried in the autumn fell on the months of February and therefore 2—3 months earlier than sowing conditions in the field permit in the temperate climatic regions with a more severe climate. Seeds which should germinate in May, after the period of late spring frosts, would require stratification not earlier than in the first part of January, storing the seeds in the 3 months preceding stratification under conditions that would not lower the moisture content and viability. Holmes and Buszewicz (3) report an articulate example of the killing of 61% of horse chestnut seeds by storing them inappropriately, in a loose condition, even though the temperature was relatively low, and never went beyond the limits 4.5°—10°C.

So far the minimal length of the time needed under conditions of low temperature for the breaking of dormancy was not known for horse chestnut (1). Since in these studies it was found that at a temperature of 20°C the readiness of stratified seeds to germinate can be demonstrated long before the seeds kept stratified at the low temperature start to germinate, this problem was given special attention. It turned out that the number of seeds ready to germinate increased with the time of stratification in cold temperatures. Already the shortest, 14-day stratification of fresh seeds at a temperature of 5°C permitted germination of a small percentage of the seeds on raising the temperature to 20°C (fig. 9). Those stratified at 5°C for a period of 98—112 days on transferring to 20°C germinated most abundantly and rapidly. During this period the seeds did not germinate in spite of the fact that they have reached full readiness to commence active growth. At the most the first germinating radicles emerged. For the practical utilization of this finding it would be necessary to move that date of initiating stratification to the end of January, when a May sowing is anticipated. At the same time the storage period before stratification would have to be extended for almost 4 months.

Making use of the suggestions presented by the authors of the Woody-Plant Seed Manual (1), the storage of horse chestnut seeds in sealed bottles at lowered temperatures (from 1° to 5°C) was employed in this study. During the 1963/64 season seeds were stored in this way partly dried to 38% moisture content for 100 and 190 days. In the next season the seeds were stored fresh at a moisture content of 48.6% for 187 days, that is to the latter part of April. It turned out that already the 100 day storage of partly dried seeds at a temperature of 3°C permitted a quick germination after stratification of the seeds at a temperature of 10°C. In the experiment with the 190 and 187 day storage periods various stratification temperatures were tried ranging from 10° to 20°C, and the 20°C temperature proved most favourable. The seeds germinated in it very quickly and in 100%, with the delay in epicotyl emergence not exceeding 10 days.

In the 1964/65 season various temperatures were used for the storage of the seeds in bottles, closed with polyethylene sheets, ranging from 1°—5°C. The highest viability of the seeds (96.5%) was obtained through storage under 1°C. Also it was found that the storage of the horse chestnut seeds in the fresh, undried condition, in sealed bottles at a temperature of 1°C provides the optimal condition for rapid and immediate germination of the seeds. On completion of the storage and removal of the seeds from the bottles it is sufficient to place the seeds in moist conditions with good aeration (e.g. stratification medium of sand and peat) at a temperature of 20°C or to sow them out shallowly in moist soil in the period when the late frosts can no longer damage the young seedlings and the soil is sufficiently warm, in order to obtain a massive germination of roots and the growth of the epicotyls.

May (5) obtained similar results. In the years 1960—1962 he found that the best way of storing seeds of Aesculus hippocastanum and A. glabra is to keep them in polyethylene bags at —0,5°C. In May's experiment the seeds germinated immediately, and in a high percentage after 130 and 175 says of such storing. The results of the present author, confirm those of May which enhences the value of them both.

It needs to be stressed here, that the storage of the seeds of horse chestnut in closed containers was considered by the authors of the Woody-Plant Seed Manual (1) and originally also by the present author as a method of protecting the seeds from a harmful desiccation. However, the fact that the seeds germinated immediately after they were transfered into favourable warm temperatures from the storage conditions of arround 0°C where they were kept for a sufficiently long time, indicates that the storing plays a role beyond that which was expected. On the basis of these results it is necessary to revise the generally accepted views on the conditions of storing and stratification of seeds from some species of trees and shrubs. There is no doubt that the storing of the seeds under low temperatures in sealed bottles has created conditions for the breaking of dormancy, the more so since both the fresh and partly dried seeds had a high moisture content (49% and 38%). Hydration of the embryonic tissues was sufficient for the physiological processes needed to prepare the seeds for germination and for germination itself to be initiated and brought to completion. On the other hand the basic principle of seed storage was that metabolic processes need to be inhibited by a lowering of the moisture content. Schönborn (7), who reviews the bulky literature concerning the storage of tree seeds, points out the fact that some species of decidous trees with large seeds do not stand a lowering of the seed moisture content without injury. Acorns and some nuts are typical examples according to Schönborn. Holmes and Buszewicz (3) include horse chestnut seeds in this group also. These can be prepared for germination by stratification at low temperature. It has to be stressed that one of the purposes of stratification is to provide sufficient access of air to the stratified seeds.

On storing the horse chestnut seeds in sealed containers at a lowered temperature both the rules for storing (lowering of moisture content) and for stratification (sufficient gaseous exchange with the outside air) were not adhered to. The absence of the stratifying medium is not an important factor since the tight sealing of the bottles and the high original moisture content of the seeds makes it unnecessary to supply the seeds with water and maintain a sufficiently high humidity level. The high moisture content of the seeds themselves is maintained and a lowered temperature permits the breaking of dormancy.

Summarizing the results obtained the following two observations need to be stressed:

1. The placing of horse chestnut seeds either fresh or with a moisture content lowered by 1/5, for a period of 100 or 190 days in sealed, tightly packed containers without any stratifying medium, at any temperature in the range 1°—5°C, permits the completion of all the physiological changes that condition the breaking of dormancy and the preparation of seeds for rapid germination, on transfer to a moist, well aerated environment at a temperature higher than 10°C. Optimal temperature for germination is 20°C.

2. The storage of horse chestnut seeds in sealed bottles at a lowered temperature plays the role of stratification except that there was no medium, and possibility of gaseous exchange with the outside air.

It is not unlikely that this find opens some new possibilities in the field of storing and preparing for germination of the seeds of some tree species. A further investigation of this problem is needed concerning the intensity of gaseous exchange between the seeds and the spaces between seeds in the storage bottles.

CONCLUSIONS

1. Freshly collected seeds of horse chestnut (*Aesculus hippocastanum* L.) with a moisture content of about 50% of fresh weight or seeds partly dried after collection to a moisture content of 38%, require for the breaking of dormancy and the germination of first roots respectively 112—126 and 84—98 days of stratification at a temperature of 5°C (optimal temperature). The germination of so treated seeds at a temperature of 5°C extends itself over a period of 7 weeks.

2. The rapid germination of seeds that were stratified immediately after collection can be assured by 98–112 days of stratification at a temperature of 5°C and then on the germination of the first roots transfer to a stratification temperature of 20°C.

3. The partial drying of the freshly collected horse chestnut seeds to the moisture content given above has no negative influence on their viability.

4. Fresh or partly dried seeds can be stored in containers filled to capacity and tightly sealed at a temperature of 1°–5°C. Fresh seeds must be stored in containers sealed with double polyethylene sheets (2 × 0.1 mm). Optimal storage conditions are provided by the temperature of 1°C.

5. During the storage of horse chestnut seeds in sealed containers at a low temperature (1°–5°C) physiological changes take place in the seeds resulting in the breaking of dormancy and a complete preparation of the seeds for germination. Such storage replaces the need for stratification.

6. Seeds stored for 100 days under the conditions specified above are fully prepared for germination and this readiness does not undergo any change when the seeds are stored for 190 days, both in the case of fresh seeds and in seeds partly dried. This readiness demonstrates itself almost immediately on transfer of seeds to germinating conditions at 20°C.

7. Seeds stored in sealed containers at a lowered temperature germinate in a high percentage when sown into the ground. The time of emergence of the epicotyls above the ground and the germinability are dependent on the soil temperatures.

LITERATURE

1. Anon — 1948. Woody-Plant Seed Manual. U.S. Department of Agriculture. Misc. Publ. No 654.

2. Come D. — 1962. Étude expérimentale de la diffusion de l'oxygène et du gaz carbonique á travers les enveloppes séminales. J. Res. Cent. nat. Res. sci., No 59 : 187–194 (ex Hort. Abstr. 1964, 34, No 3927).

3. Holmes G. D., and Buszewicz G. — 1958. The storage of seed of temperate forest tree species. Forestry Abstracts, 19 (3) : 313–320 and (14) : 455–476.

4. Krüssmann G. — 1964. Die Baumschule. Paul Parey, Berlin.

5. May C. — 1963. Note on storage of buckeye and horse chestnut seed. The American Horticultural Magazine, 42 (4) : 231–232.

6. Schönborn A. — 1964. Die Aufbewahrung des Saatgutes der Waldbäume. Bayerischer Landwirtschaftsverlag, München.

7. Tyszkiewicz S. — 1948. Nasiennictwo Leśne. Inst. Bad. Leśn., ser. D, nr 2.

BOLESŁAW SUSZKA

Warunki ustępowania spoczynku i kiełkowania nasion kasztanowca białego (Aesculus hippocastanum L.*)*

Wnioski

1. Świeżo zebrane nasiona kasztanowca zwyczajnego (*Aesculus hippocastanum* L.) o wilgotności około 50% (w świeżej masie) lub nasiona podsuszone po zbiorze (wilgotność 38%) wymagają dla ustąpienia spoczynku i pojawienia się pierwszych kiełkujących korzeni odpowiednio: 112—126 lub 84—98 dni stratyfikacji w temperaturze 5°C (temperatura optymalna). Kiełkowanie tak traktowanych nasion rozciąga się w temperaturze 5°C na okres około 7 tygodni.

2. Szybkie i energiczne kiełkowanie nasion stratyfikowanych bezpośrednio po zbiorze można zapewnić przez 98—112 dni stratyfikacji w temperaturze 5°C i podwyższenie temperatury stratyfikacji do 20°C w momencie pojawienia się pierwszych kiełkujących korzeni.

3. Podsuszenie świeżych nasion kasztanowca do podanego powyżej poziomu wilgotności nie wywiera żadnego ujemnego wpływu na stan ich żywotności.

4. Nasiona świeże lub podsuszone można przechowywać w całkowicie wypełnionych, szczelnie zamkniętych zbiornikach w temperaturach 1°—5°C. Optymalne warunki przechowania zapewnia temperatura 1°C. Nasiona świeże muszą być przechowywane w zbiornikach zamkniętych tylko podwójną warstwą folii polietylenowej (2 × 0,1 mm).

5. Podczas przechowywania nasion kasztanowca w zamkniętych zbiornikach i obniżonej temperaturze (1°—5°C) zachodzą procesy powodujące całkowite ustąpienie spoczynku i osiągnięcie gotowości do skiełkowania. Przechowanie takie zastępuje zabieg stratyfikacji.

6. Nasiona przechowane w podanych powyżej warunkach przez 100 dni są w pełni przygotowane do kiełkowania, gotowość ta nie ulega zmianie przy 190 dniach przechowywania tak nasion świeżych o wysokim poziomie wilgotności, jak nasion podsuszonych. Ujawnienie tej gotowości następuje prawie natychmiast w temperaturze 20°C.

7. Nasiona przechowywane w zamkniętych zbiornikach w obniżonej temperaturze kiełkują po wysiewie do gruntu w wysokim procencie. Szybkość pojawiania się pędów po wysiewie do gruntu i zdolność kiełkowania zależna jest od przebiegu temperatury glebowej.