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## CONDITIONS FOR THE BREAKING OF DORMANCY AND GERMINA-TION OF HORNBEAM (CARPINUS BETULUS L.) SEEDS

(Warunki ustępowania spoczynku i kiełkowania nasion grabu zwyczajnego (Carpinus betulus L.))

#### Introduction

Until recently it has been believed that mass germination of hornbeam seeds the first spring following collection can only be obtained by immediate sowing (Tyszkiewicz 1949) or by the stratification of nutlets (Krüssmann 1954) collected before they have reached full maturity, namely at the end of August or the beginning of September. Seedlings from nutlets collected in the condition of incomplete ripeness or fully ripe in October when sown immediately after collection or after winter stratification emerge the following spring in a very low percentage. Usually they remain dormant until the second spring (Tyszkiewicz 1949, Tyszkiewicz and Dąbrowska 1953, Winkler 1955, Jahnel 1956, Zentsch 1961). For this reason it has been generally recommended that stratification of seeds collected in the fully ripe condition be started in the spring following winter storage in a partially dried condition. After a full year of stratification in the natural thermal conditions such seeds can be sown in the second spring after collection (Tyszkiewicz 1949, Tyszkiewicz and Dąbrowska 1953, Krüssmann 1954).

Duration of the cold stratification necessary to precondition hornbeam seeds for germination has been determined by Jahnel (1956) as 24–26 weeks. In the last 8 weeks of this period he recommends that the stratification temperature be lowered to almost 0°C in order to inhibit the emergence and growth of roots. In Poland it has been found (Tyszkiewicz and Dąbrowska 1953) that immediate stratification of green hornbeam nutlets, collected in the first 10 days of October, resulted in the germination of 700/<sub>0</sub> of seeds before the end of the stratification, which lasted until mid-April of the following year. After sowing seedlings have emerged in a very low percentage. Brown nutlets collected simultaneously with the green ones and treated in the same way have germinated in 290/<sub>0</sub> and after sowing have

emerged very poorely. Seeds from the same collection stratified two months later did not germinate during stratification at all and after sowing the seedlings did not emerge. In other experiments of Tyszkiewicz and Dabrowska (1953) nutlets collected between the 10th and 20th of October and sown immediately have vielded seedlings in 21% during the spring and when stratified after collection in boxes in a stratification pit and sown in March have vielded seedlings up to 38%. During stratification of nutlets from the same collection in a cellar it turned out that in March on completion of the stratification period  $90^{0}/_{0}$  of seeds had overgrown radicles. When these seeds were sown in the same month there was almost no emergence of seedlings at all. Still other lots of nuts also collected in mid-October and sown immediately in the ground have yielded almost no seedlings  $(0.3^{\circ}/_{0})$  in the following spring but in the spring a year later seedling emergence was massive. Following stratification in a pit, started one month after collection, a small percentage seedlings emerged toward the end of March  $(3-6^{\circ})_{\circ}$  depending on the thermal conditions of the cold stratification). From the studies of Tyszkiewicz and Dabrowska it appears that a 23 week period of stratification for the nutlets of hornbeam is too long. In the case of nutlets stratified immediately after collection these authors have stratified them in warm conditions for one month in an unheated room, prior to burying the boxes into the soil. Results obtained by them in these variable conditions of temperature have led them to formulate the following conclusion: The earlier are the nuts of hornbeam collected and the milder are the temperature conditions in which the stratification is begun then the earlier will they be ready for germination and therefore may germinate too early, already in very early spring in the stratification pit.

Jahnel (1956) has employed in his studies (conducted in the German Democratic Republic) a stratification at the following temperatures:  $-3^{\circ}$ ,  $1^{\circ}$ ,  $4^{\circ}$  and  $7^{\circ}$ C. Seeds have germinated only after stratification at  $4^{\circ}$  and  $7^{\circ}$ C, and only then when in the early phase of stratification the temperature due to faults in the apparatus has risen to room conditions.

Vincent (1959) has found in Czechoslovakia that the most favorable way of preconditioning hornbeam seeds for germination is by applying originally a raised temperature and only later low temperature lasting together 21–23 weeks. Kocięcki (1964, 1965) has studied this problem in the years 1956–1960 and has arrived at the conclusion that hornbeam nutlets collected in Poland in September or in early October, have to be soaked in water for 3 days and then stratified first for a month at 20°C and then for about 21 weeks at a temperature of 5°C. If in the final stage of such a stratification one notices the occurrence of the first germinating seeds then immediate sowing can result in up to  $90^{0}/_{0}$  of the seeds yielding viable seedlings. When sowing seeds that have germinated too early the success of the sowing declines rapidly. The only way to prevent the seeds of hornbeam from premature germination is according to Kocięcki (1965) to transfer the seeds to a room with a temperature of about 0°C.

In the Laboratory of Seed Physiology of the Institute of Dendrology and Kórnik Arboretum of the Polish Academy of Sciences studies have been conducted in the years 1963–1966 on the conditions for the breaking of seed dormancy and the germination of hornbeam seeds. In the studies particular note was taken of:

a) a comparison between the effectiveness of a cold and a warm-followed-by-cold stratification of completely ripe seeds stratified:  $1^{\circ}$  — immediately after collection,  $2^{\circ}$  — immediately after partial drying, and  $3^{\circ}$  — after partial drying and storage for one year;

b) determination of the duration of the process of seed after-ripening;

c) determination of the optimal temperature for the germination of stratified seeds;

d) a comparison of the emergence of seedlings from seeds sown after stratification in controlled thermal conditions and in the field conditions.

## Materials and methods

**Material:** For the study use was made of seeds in completely ripe nutlets, brown in color, collected in the first 10 days of November. The seeds came from trees growing in the Kórnik Arboretum. The experiments were conducted simultaneously on several lots of seeds, representing individual trees. The nut covers (wings) have been removed both for the stratification and for the storage of nutlets.

**Partial drying of the nuts and the determination of water content:** The nutlets have been partialy dried at a temperature of 15–18°C., and water content has been determined by weight after drying for 2 days at 105°C. The values given in the paper represent the percentage water content in the fresh weight of the nutlets.

Determination of seed viability: Seeds viability was estimated by the cutting test.

Seed storage: Nutlets have been stored for one year after partial drying and dewinging in tightly closed jars at a temperature of 3°C.

Stratification: Nutlets have been stratified in a moist mixture of sand and sieved peat moss (1:1 by volume). Moisture of the mixture has been checked at 2-week intervals during the warm stratification and during the cold stratification depending on the experiment every 2 or 3 weeks. Warm stratification has been conducted at 20°C and cold stratification at 1°, 3°, 5° and 10°C. Temperature fluctuations did not exceed  $\pm 0.5^{\circ}$ C.

Seedling emergence in the laboratory conditions: In the years 1965 and 1966 seedling emergence tests have been conducted in plastic boxes  $20 \times 20$  cm filled with a moist mixture of sand and peat. Nutlets have been divided after stratification into the following categories: whole nutlets, cracked nutlets and nutlets with a visible

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radicle (root) not exceeding 3 mm in length. Seeds of individual categories have been sown singly into holes 1 cm deep, which have been made in the medium, by a perforated metal form. After sowing these holes were filled with dry sand using for the purpose the same forms. Then the medium in the boxes was moistened by sprinkling with water, which has been applied in the same way whenever necessary throughout the duration (24 days) of the test. Every third day the number of emerged seedlings was recorded and the seedlings were removed. On completion of the emergence test all the ungerminated seeds were subjected to the seed viability test. The seedling emergence test was conducted at a temperature of 20°C.

Seedling emergence in the field conditions: In 1966 seedling emergence tests have been conducted also in the field conditions. For this purpose seeds were used from the same lots as those which in the same year have been used for the emergence tests in the laboratory conditions. Nutlets have been sown in the nursery in furrows 120 cm long (width of the bed) and 1 cm deep. Each furrow was one replicate of one experimental variant comprising 50 nutlets. The nutles were divided into two groups, whole and cracked. Partially germinated seeds have not been sown. After sowing the furrows were covered with a 1 cm layer of soil, which was slightly pressed. When necessary the seed beds were sprinkled with water at times of drought even twice daily. Soil temperature at the seed level (1 cm) has been checked three times a day, (at 7<sup>oo</sup>, 13<sup>oo</sup> and 21<sup>oo</sup> hrs.). Control of seedling emergence has been made every 2nd day, however the emerged seedlings have not been removed as was the case in the laboratory conditions. The whole experiment was laid out in a complete block design with 4 replications.

Number of replicates in the experiment: All the experiments on the stratification of seeds, emergence tests in the laboratory and in the field have been performed in 4 replicates, one replicate for each experimental variable containing 50 or 100 (in 1965) seeds.

## Results

The studies on the breaking of dormancy and germination of hornbeam seeds conducted in Kórnik in the years 1963–1966 covered the following topics:

1. Breaking of seeds dormancy during a cold and a warm-followed-by-cold stratification (experiments 1a and 1b).

2. Optimal germination temperature for the stratified seeds (experiment 2).

3. Reaction of the stratified seeds to a raising of the temperature (experiments 3 and 4).

4. Emergence of seedlings in laboratory and field conditions from stratified seeds (experiment 4).

#### 1. Breaking of Dormancy of Hornbeam Seeds During Cold and Warm-Followed-by-Cold Stratification

Studies on the topic mentioned in the subtitle above have been conducted over several years. In the 1963/64 season (experiment 1a) seeds collected from 2 trees have been studied separately. After collection the nutlets have been partially dried to  $10-11^{0}/_{0}$  of water content and stratified immediately after completion of the partial drying.



Fig. 1. Design of experiment la: A — seeds from tree 1, B — seeds from tree 2.

Besides the cold stratification at 1°, 3°, 5° and 10°C a warm-followed-by-cold stratification was employed. Individual variants of the experiment differed in duration of the warm phase (20°C) which was 2, 4, 6, 8, 10, or 12 weeks long. The cold phase was run at 1°, 3° and 5°C and always lasted 30 weeks (Fig. 1). Germination of hornbeam seeds has been observed during and not after the stratification.

A short characteristic of experiment la

Purpose: A comparison of the effect of cold stratification with the effect of the warm-followed-by-cold stratification on the germination of seeds during stratification. Design of the experiment: see Fig. 1.

Collection of nutlets: in the Kórnik Arboretum, Nov. 10, 1963

Partial drying of the nutlets: from Nov. 10, 1963 to Nov. 25, 1963

Water content of the nutlets after partial drying was for:

tree 1-10.0%

tree 2-10.4%/0

Seed viability after partial drying:

tree 1-92.3% of viable seeds (Nov. 25, 1963)

tree 2-75.3% of viable seeds (Nov. 25, 1963)

tree 1 - see Fig. 2

Stratification begun on: Nov. 26, 1963. Results of the experiment:



Fig. 2. Course of germination of hornbeam seeds partially dried after collection and placed directly in conditions of cold, and warm-followed-by-cold stratification (experiment 1a, seeds from tree 1).

The results obtained using seeds from two different trees were very much the same (Fig. 2 and 3). Cold stratification at a temperature of 1° and 3°C had practically no effect on the germination of the seeds and at 5°C the seeds germinated in a very low percentage. The highest germination of seeds following cold stratification has been obtained when the germination temperature was 10°C. In





contrast to the other stratification temperatures, at 10°C the seeds kept on germinating all the way until the 30th week of stratification, that is to the time when the experiment was terminated.

As a result of the warm-followed-by-cold stratification the seed germination was very different. Already a two week period of warm stratification was sufficient to hasten the onset of stratification and has helped in increasing the number of germinating seeds (Figs. 2 and 3). The best results were obtained after a 4 week warm stratification followed by a cold stratification at 5°C. Seeds kept at lower

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temperatures during the cold phase of the stratification (at 1° and 3°C) have germinated to a similarly high percentage as at 5°C but only after the first warm phase of the stratification lasted at least 6 weeks. Elongation of the duration of the warm phase of the stratification did not help in increasing the number of germinating seeds, since already in the optimal sequence of thermal conditions given above all the viable seeds have germinated. There was no negative effect of the extension of the warm phase up to 12 weeks on the germination of hornbeam seeds during the cold phase. Thus the best conditions for the stratification of hornbeam seeds, collected in late Autumn and partially dried immediately after collection proved to be: 2 or 4 weeks at 20°C followed by stratification at 5°C. First germinating seeds have been found following such treatment between the 12th and 15th week of the cold phase of the stratification, and the last around the 21st week. The period of seed germination has lasted therefore about 9 weeks.

Having determined the most effective combination of thermal conditions for the stratification of hornbeam seeds further studies (experiment 1b) have ben conducted in the next season, 1964/65 for which not only partially dried seeds but also freshly collected seeds from 3 trees have been used. In the studies a comparison was again made of the cold stratification and the warm-followed-by-cold stratification. In the warm phase 20°C was provided only for 2, 3 and 4 weeks and in the cold phase the temperatures employed were 3°, 5° and 10°C.

#### A short characteristic of experiment 1b

Purpose: To establish the effect of warm-followed-by-cold stratification with a short warm phase.

Design of the experiment: see Fig. 4.

Collection of the nuts: in the Kórnik Arboretum, Nov. 5–7, 1964 Partial drying of the nuts: from Nov. 5–7, 1964 to Nov. 20, 1964 Water content in the nutlets following collection:

tree  $3-19.0^{0}/_{0}$ tree  $4-17.1^{0}/_{0}$ tree  $5-18.0^{0}/_{0}$ Water content in the nutlets following partial drying: tree  $3-9.2^{0}/_{0}$ tree  $4-9.8^{0}/_{0}$ tree  $5-9.9^{0}/_{0}$ 

Viability of the partially dried seeds:

tree  $3-92.7^{\circ}/_{0}$  of viable seeds tree  $4-81.0^{\circ}/_{0}$  of viable seeds tree  $5-78.8^{\circ}/_{0}$  of viable seeds

Onset of stratification:

freshly collected seeds - Nov. 7, 1964 partially dried seeds - Nov. 21, 1964

Fresh seeds (not partially dried) have been stratified at only one set of temperatures (20°C. for 4 weeks and later  $5^{\circ}$ C.).

Results of the experiment: see Fig. 5.

Stratification only at the cold conditions run at  $3^{\circ}$  and  $5^{\circ}$ C has not given any results and even at  $10^{\circ}$ C hardly any seeds germinated. On the other hand, a significant effect of the warm-followed-by-cold stratification has been exerted on the breaking of dormancy of the seeds and this regardless of the temperature of the cold phase ( $3^{\circ}$ ,  $5^{\circ}$  or  $10^{\circ}$ C.) (Fig. 5). Under such stratification conditions



Fig. 4. Design of experiment 1b: A — fresh seeds, B — partially dried seeds

all viable seeds from trees 3 and 5 have germinated. The germination gradient of tree 4 has not reached its culmination in the time period employed in the experiment. Individual variation has shown itself here in the uneven reaction of seeds from individual trees on the same thermal conditions of stratification. The course of seed germination from the individual trees was dependent on the temperature of the cold phase of the stratification. Germination at 5°C was earliest while the seeds kept at 3°C germinated 2–4 weeks later. At 10°C germination was latest, however the germinative capacity was highest at this temperature of the cold phase of the stratification.

Seeds stratified immediately after collection (17-19%) of water) in the con-

ditions: 4 weeks at 20°C followed by 5°C, have germinated in the case of two trees (three were studied) to a higher percentage than seeds partially dried.

Seed germination occurred in the period between the 12–14th and 26–30th week of the cold phase of stratification. This implies that between the germination of the first and last seeds a period of about 15 weeks has elapsed.



Fig. 5. Course of germination of hornbeam seeds stratified immediately after collection (5°A) or after partial drying in conditions of cold and warm-followed-by-cold stratification (experiment 1b, seeds from trees 3, 4 and 5).

From the results of experiment 1b it appears that only the warm-followed-bycold stratification of hornbeam seeds, partially dried or fresh, guarantees the germination of the bulk of the viable seeds. The warm phase of this treatment should last for about 2–4 weeks at 20°C, after which it is necessary to lower the temperature to 5°C. In the period between the 12th and 16th week of the cold phase one is to expect the first germinating seeds. These results constitute a confirmation of the results obtained in the 1963/64 season.

#### 2. Optimal Temperature for the Germination of Stratified Seeds of Hornbeam

In the experiments 1a and 1b the germination of hornbeam seeds during stratification has been studied. As it turned out at a relatively low temperature of the cold phase of this treatment the period covering the germination of all the healthy seeds lasted for a long period of time and may reach as much as several weeks. When sowing the seeds in the field conditions as well as in the laboratory, the aim is to obtain the fastest possible germination of the seeds. In experiment 2 established with this aim in mind an attempt was made to define the conditions in which it would be possible to obtain an energetic germination of the greatest number of seeds in the shortest possible time.

#### A short characteristic of experiment 2

A larger portion of hornbeam nutlets from tree No. 3 have been stratified in the fall of 1964, immediately after completing the partial drying, under conditions of the optimal warm-followed-by-cold stratification. During the germination period the seeds were divided into those already germinating with a radicle not longer than 3 mm, those still ungerminated but in cracked nutlets and those in whole nutlets. The germinators with seeds of each category have been placed at temperatures  $5^{\circ}$ ,  $10^{\circ}$ ,  $15^{\circ}$  and  $20^{\circ}$ C. The course of seedling emergence is shown in Fig. 6.

From partially germinating seeds seedlings have emerged in  $100^{0}$  regardless of the temperature which acts only on the rate of the emergence. At the optimal temperature of 20°C all the seedlings have emerged between the 4th and 12th day of the test. Seeds of this category have yielded seedlings at the lowest rate and therefore latest at temperature 5°C namely between the 12th and 48th day of the test (Fig. 6A).

Seeds from cracked nutlets have differed from the partially germinated seeds in that at all temperatures the seedlings emerged later. At temperatures of 20°,  $15^{\circ}$  and 10°C seedling emergence has reached  $100^{0}/_{0}$ . The seeds sown at 5°C have yielded seedlings latest, between the 30th and 58th day of the test, and only in 80<sup>0</sup>/<sub>0</sub>. At the optimal temperature of 20°C the seedlings have emerged between the 4th and 16th day of the test (Fig. 6B).

Nutlets of the first two categories have contained only viable seeds. On the other hand the whole nutlets were to a large percentage empty or contained non-

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Fig. 6. Emergence of seedlings from hornbeam seeds at temperatures 5°, 10°, 15° and 20°C. Seeds were sown after warm-followed-by-cold stratification to a depth of 1 cm (experiment 2, seeds from many trees):

A — partially germinated seeds, B — seeds in cracked nutlets, C — sound seeds from complete nutlets

viable seeds. The numerical values drawn in the graphs for the whole nutlets (Fig. 6C) have been calculated as a percentage of the viable seeds as determined on the day of termination of the experiment. As it turned out the seeds from whole nutlets have germinated even later than those from the other categories. They germinated earliest at 20°C, between the 6th and 16th day of the test. The germination capacity of the seeds from whole nutlets was lowered. It was highest  $(950/_0)$  at 10°C and lowest  $(400/_0)$  at 5°C.

Seeds in cracked nutlets were as it turned out completely ready for germination. Thus cracked nutlets of hornbeam can be sown at a temperature of  $20^{\circ}$ C and in the field conditions at such time when the soil at sowing depth has a similar temperature. At the raised temperature such seeds do not fall into a secondary dormancy.

#### 3. The Reaction of Stratified Hornbeam Seeds to the Raising of Temperature

A temperature of 20°C is optimal for the germination of stratified seeds of hornbeam. It is known however from the studies conducted on the seeds of other genera (Nikolaeva 1963, Suszka 1967) that even the best conditions for germination can contribute to the return of the seeds into the dormant condition (secondary dormancy). This can take place when not all the seeds have been preconditioned for germination by the stratification. In some species the seeds reach a condition of readiness for germination under appropriate conditions even when this readiness is not manifest in any way externally (Suszka 1966). In the case of seeds investigated in the present study it was considered necessary to establish the shortest stratification period after which by raising the temperature one would obtain a rapid and energetic germination of the highest percentage of viable seeds.

#### A short characteristic of the experiment 3

Purpose: To determine the shortest stratification period for the seeds of hornbeam.
Design of the experiment: see Fig. 7.
The material: seeds collected from tree 3 in the Kórnik Arboretum.
Collection of nutlets: Nov. 7, 1964.
Partial drying of the nutlets: from Nov. 7, 1964 to Nov. 19, 1964.
Water content after partial drying: 9.2%
Seed viability after partial drying: 92.7% of viable seeds.
Stratification begun: immediately after partial drying, Nov. 19, 1964.
Results of the experiment: see Fig. 8.
In experiment 3 the stratification temperature was changed twice. The first change took place in all the variants after 4 weeks, at the moment when warm (20°C) phase of the stratification was completed and the cold (5°C) phase begun.

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The different variants of the experiment varied in the duration of the cold phase (2, 4, 6, 8, 10, 12, 14 or 16 weeks). After the cold stratification the temperature

was again raised to 20°C and maintained so for 48 days. This period was treated as a germination test and control observations have been made during the 48 days at 6 day intervals.

Results of the experiment are presented in Fig. 8. The germination was first noticed in a very small percentage after a stratification comprising 4 weeks of warmth at 20°C followed by 8 weeks of the cold stratification at 5°C. With the extension of the cold phase of the stratification the percentage of seeds that have germinated, after having raised the temperature, has risen quickly reaching a



Fig. 7. Design of experiment 3.

maximal value after stratification comprising 4 weeks of warm (20°C) temperature and 14 weeks of cold (5°C) temperature. In this time, that is after 14 weeks of the cold phase of the stratification, it was also possible to notice the first seeds germinating already at the 5°C temperature of the stratification prior to the raising of the temperature. A further extension of the cold period has resulted in the increase of the number of germinating seeds already at the 5°C which in the case of hornbeam has to be considered as undesirable. Seeds that germinated too far generally do not emerge even after very shallow sowing.

Starting from the 4+12 weeks of the warm-followed-by-cold stratification seeds germinated after raising of the temperature rapidly, within 6 days. It would appear



Fig. 8. Germination of hornbeam seeds at temperature 20°C following warm-followed-by-cold stratification. Individual experimental variants differ in the length of the cold phase of stratification. The germination test lasts 48 days (experiment 3, seeds from tree 3).

that the rate of germination was higher here than in the case of experiment 2 which however was not so. In the latter experiment seedlings were observed which had to pierce a 1 cm layer of sand after germination.

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On the basis of results of the experiment discussed here it can be stated that the time of germination of the first seeds of hornbeam observed after 12–14 weeks of the cold phase of the warm-followed-by-cold stratification (4 weeks at  $20^{\circ}C$ + +12–14 weeks at 5°C) determines the most satisfactory time for the sowing of the seeds out at a temperature of 20°C. Almost all the seeds at this time are ready for germination. Under such thermal conditions the germination takes place very quickly, within a few days.

# 4. A Comparison of the Emergence of Hornbeam Seedlings from Seeds First Stratified in the Warm-followed-by-cold Conditions and then Sown in the Laboratory and in the Field

Results of the laboratory tests require a confirmation under field conditions. For these reasons in the 1965/66 season the following experiment was conducted:

#### A short characteristic of experiment 4

Purpose: A comparison of seedling emergence from seeds sown simultaneously in controlled conditions at 20°C and in the field following a warm-followed-by-cold stratification.

Material: the nutlets have been collected in November 1965 in a state of complete ripeness, separately for 5 trees, partially dried after collection to  $9-10^{0}/_{0}$  of water content and stored in tightly sealed jars at a temperature of 3°C for 13-14 months. Water content in the nutlets and seed viability after completion of the storage: (N. B. — the values found before the onset of storage are given in brackets)

	Water content as $0/0$ of the fresh weight	Seed viability
tree No. 3	8.8% (9.2%)	85.7% (92.7%)
tree No. 4	9.2% (9.8%)	78.8% (81.0%)
tree No. 5	9.2% (9.9%)	76.3% (78.8%)
tree No. 6	9.5%	74.0%
tree No. 7	10.0%	88.7%

Design of the experiment: in the experiment the following variants of the warm-followed-by--cold stratification have been used:

A) 4 weeks at 20°C+10 weeks at 5°C (strat. begun Jan. 24, 1966)

B) 4 weeks at  $20^{\circ}C + 12\frac{1}{2}$  weeks at 5°C (strat. begun Jan. 6, 1966)

C) 4 weeks at 20°C+15 weeks at 5°C (strat. begun Dec. 20, 1965)

Seeds of the individual trees have been stratified in the following variants of the duration of the stratification: tree 3 - B; tree 4 - A, B, C; tree 5 - A, B, C; tree 6 - A, B; and tree 7 - B.

Stratification at all the time variants (A, B, C) has been terminated simultaneously on May 3, 1966 after which the seeds of each stratified portion have been divided into the following categories of seeds: germinated seeds, ungerminated seeds in cracked nutlets, and seeds in whole nutlets. Each lot has been divided into 4 replicates with 50 seeds, keeping the 3 categories within each replicate in the same proportions. In the laboratory sowings as well as in the field the same sowing depth of 1 cm was employed.

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Conditions of the seedling emergence test in the laboratory: a dark room of the phytotron at  $20^{\circ}$ C, control observations every 3 days for 24 days.

Conditions of the emergence test in the field: The thermal conditions at the sowing depth — see Fig. 9. The field sowings were performed in the complete block design with 4 replicates. Control observations were made every 2 days for 35 days.

Results of the experiment: see Fig. 10.



Fig. 9. Soil temperature at the sowing depth (1 cm) in the period from May 3, 1966 to June 8, 1966 (experiment 4).

In Fig. 10 the course of the seedling emergence is presented for only one of the three experimental variants (variant B). In this variant the seeds were sown in the laboratory and in the field following a stratification for 4 weeks at  $20^{\circ}$ C and  $12^{1/2}$  weeks at 5°C. The results obtained in the remaining variants (with 10 and 15 weeks at 5°C) have not been presented graphically since they do not include seeds from all the 5 trees. From the graph of temperature changes in the soil at a depth of 1 cm it appears (Fig. 9) that the mean daily temperature fluctuations varied during the seedling emergence test between 8° and 22°C. Extremal soil temperatures during the period concerned were 7.2° and 34.5°C.

A warm-followed-by-cold stratification with a 10 week cold phase (variant A) was insufficient. Even in the laboratory conditions only 50–70% of seedlings have emerged from the viable seeds from cracked and whole nutlets. A stratification for 15 weeks at 5°C (variant C) was too long since to a large extent the seeds have germinated before the termination of the stratification. The intermediate variant (B) of 4 weeks at 20°C+12<sup>1</sup>/<sub>2</sub> weeks at 5°C proved most satisfactory since at the time of termination of the stratification in 4 of the studied 5 lots of seeds only small percentage of seeds has germinated. In laboratory conditions, that is at a temperature of 20°C after such a stratification from all or almost all the cracked nutlets



Fig. 10. Emergence of seedlings of hornbeam from seeds sown under controlled conditions at 20°C and in the field conditions following 14 months of storage in a partially dried condition in sealed containers at 3°C and a warm-followed-by-cold stratification (4 weeks  $20^{\circ}C+$ +12.5 weeks 5°C). State of the seeds on the day of sowing: a - germinating seeds, b - seeds in cracked nutlets, c - seeds in whole nutlets (experiment 4, seeds from trees 3, 4, 5, 6, and 7).

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seedlings have emerged. It needs to be pointed out that it is this category of seeds which was in all the seeds lots after the  $4+12^{1/2}$  weeks of stratification most numerous. Viable seeds from the whole nuts have emerged in 50–100<sup>0</sup>/<sub>0</sub> excluding the nutlets which after termination of the experiment were found to be empty. The seedling emergence had an energetic course. Most have emerged in the period between the 6th and 15th day of the laboratory test.

In the field experiment the seedlings have emerged somewhat later, in the period between the 17th and 30th day from sowing. Germinative capacity was lower compared with the laboratory conditions by about  $30^{0}/_{0}$ . This was caused by about  $25-30^{0}/_{0}$  of the seeds from cracked nutlets and about  $50^{0}/_{0}$  of viable seeds from whole nutlets failing to germinate in the field conditions. Thus in the field conditions about  $70^{0}/_{0}$  of the viable seeds have yielded seedlings.

### Discussion

Seeds of hornbeam germinate in the natural conditions on the second spring following the seed fall if they happen to find themselves in appropriate conditions permitting after-ripening. Such conditions occur in the upper layer of the moist soil, covered in the summer by litter and in the winter also by the snow. Thanks to this cover the soil remains for the greater part of the year moist and the daily temperature fluctuations are somewhat buffered by these conditions. From the studies presented in this paper it appears that during the cold stratification at temperatures between 1° and 5°C the seeds will remain to a large percentage in the dormant condition and only at a temperature of 10°C some of the seeds will germinate slowly and very late. However if the cold stratification is preceded by a warm stratification the seeds will germinate in a large percentage.

The treatment of the warm-followed-by-cold stratification reproduces somewhat the natural course of events in respect of the thermal conditions existing in the upper layer of the moist forest soil covered by litter during summer, autumn and winter. For these reasons recommendations were made for several years that stratification of hornbeam nutlets be started in late spring (Tyszkiewicz 1949, Winkler 1955, Kocięcki 1965). The need of stratification of hornbeam seeds at a raised temperature first and then later at a low temperature has been pointed out by others (Tyszkiewicz and Dąbrowska 1953, Vincent 1959) but the conditions were first proposed specifically by Kocięcki (1964, 1965) recommending that after an October collection at first for about 4 weeks the nuts be stratified at 20°C and then for 5 months at about 5°C.

In the light of our studies various doubts about the time of seeds collection and the time of stratification expressed by several authors have been removed. It was shown that one can completely abandon the collection of hornbeam nutlets "green", because even following the latest collection in the autumn the seeds will germinate the following spring in a large percentage if the conditions of stratification and sowing as developed in the present study are supplied. Also it is not necessary to store the seeds over the winter and to stratify them in May or June, since already a 2-week period of warm stratification is sufficient to guarantee satisfactory results provided that the warm period is followed by a cold stratification at 5°C. Other temperatures of the cold phase of stratification do not affect the germinative capacity of the seeds, which is always high within the 1–10°C range of temperatures, but the time of germination. In temperatures higher or lower than 5°C the germination occurs generally several weeks later.

The first, that is the warm phase of stratification can be extended even to 12 weeks (the longest period studied) without any loss of seed viability and germination capacity. In natural conditions the warm period of stratification lasts in the soil even for a longer period of time, covering a large part of the spring, all the summer and much of the autumn.

The optimal duration of the stratification, described by different authors as lasting 21–26 weeks (Jahnel 1956, Vincent 1959, Kocięcki 1965) can be considerably shorter. It turned out in our studies that the warm-followed-by-cold stratification with a 2–3 week warm period followed by 12–14 weeks of cold stratification preconditions almost all the seeds to germination.

Having determined the optimal conditions for the germination of hornbeam seeds to be 20°C studies were conducted on the raising of the temperature of the cold phase of stratification from 5°C to 20°C. From studies on seeds of several species from the genus *Acer* and *Evonymus* (Nikolaeva 1963) or *Prunus* (Suszka 1967) it appears that the sudden raising of temperature of the cold stratification can cause a massive return to the dormant state of the seeds which have already been partially preconditioned for germination. In the case of hornbeam however it was found here that the readiness of seeds in the same condition for germination is best demonstrated at the raised temperature of 20°C and an induction into secondary dormancy does not occur. The conditions when almost all the seeds can on raising the temperature to 20°C germinate energetically (2–3 weeks at 20°C+12–14 weeks at 5°C) are signalized in the cold phase of the stratification by the occurrence of the first germinating seeds. Seed germination, which during the stratification normally begins at about the 12–14th week of the cold phase and lasts on the average for about 9–12 weeks, is shortened to only a few days by raising the temperature.

By maintaining the conditions described above it is possible to bring completely dormant seeds into massive germination within about 15 weeks of a warm-followed-by-cold stratification. Field sowings made parallel with the laboratory tests have confirmed the results obtained in the controlled conditions. The time of seed germination in the field is dependent on the soil temperature at the sowing depth. The closer will the temperature of the soil at the time of sowing be to the optimum (20°C) the closer will the result in the field be to the results obtained in the laboratory conditions. Under controlled conditions at 20°C almost all the seeds germinated and in the field conditions only  $70^{0}/_{0}$  of that number. An important con-

dition for the success of the sowing is the depth as shallow as possible to which the seeds are sown, and it should not exceed 1 cm.

In the experiments for which stored seeds have been used it turned out that the storage of dewinged, partially dried nutlets in tightly sealed containers at a temperature of 3°C for 14 months did not contribute to the lowering of seed viability and does not lower in any way the number of seeds that germinated under appropriate conditions.

## Conclusions

1. Nutlets of hornbeam should be collected in the conditions of full ripeness. Collecting earlier is not to be recommended.

2. Drying of the nutlets to a water content of  $9-11^{0}/_{0}$ , performed immediately after collection at room temperature or lower, has no negative effects on the viability and germinability of the seeds. It makes no difference whether the stratification of partially dried seeds is begun immediately after drying or whether it comes after a year of storage in closed containers at low temperature (3°C or lower).

3. Freshly collected nutlets  $(17-19^{0}/_{0} \text{ of water})$  or nutlets partially dried after collection  $(9-11^{0}/_{0} \text{ of water})$  stratified immediately after collection or a year later following storage of the partially dried seeds, will germinate in the highest percentage when subjected to a warm-followed-by-cold stratification. Such a stratification has to comprise a 2-4 week phase of warm stratification at 20°C followed by a cold phase at 5°C. Under these conditions germination will begin in the 12-14th week of cold stratification. The period from the germination of the first to the last seed lasts for seeds of most trees arround 9 weeks at 5°C.

4. Only cold stratification at temperatures of 1°, 3° and 5°C is not very effective for the seeds of hornbeam. However at 10°C seeds of some of the trees germinate in a large percentage. The course of germination at 10°C is slow and the onset is much delayed.

5. Temperatures 1°, 3°, 5° and 10°C employed in the cold phase of the warm-followed-by-cold stratification are for most of the trees equally and highly effective. Seeds at 5°C germinate earliest and most energetically, while seeds at 10°C germinate latest.

6. Prolongation of the warm phase of the warm-followed-by-cold stratification to 12 weeks (the longest period studied) does not lower the viability and germinative capacity of the seeds.

7. Seeds of hornbeam germinate most energetically when the temperature of the cold phase of stratification is after 12–14 weeks raised to 20°C. At this temperature within 6–8 days all the partially germinated seeds will germinate as well as all the seeds from cracked nutlets and most of the healthy seeds from uncracked nutlets. Use of temperatures lower than 20°C causes delay in the onset of germination and lowers its rate. Shortening of the cold phase of stratification lowers pro-

gressively the germinative capacity of the seeds at the raised temperature. On the other hand extension of the cold stratification beyond 12–14 weeks results in the germination of ever increasing number of seeds before the raising of the temperature, which is not required.

8. Optimal for seed sowing under conditions of raised temperature is the period of the first appearance of germinating seeds still in the cold phase of stratification. Under the optimal conditions of warm-followed-by-cold stratification (at  $20^{\circ}/5^{\circ}$ C) this period comes after 12–14 weeks of the cold period. Almost all the seeds are ready for germination at the time and the raising of temperature will not induce secondary dormancy.

9. Warm-followed-by-cold stratification of hornbeam seeds guarantees the highest yield of seedlings also under field conditions, following sowing in the ground in the period when daily mean soil temperatures at the sowing depth (1 cm) vary between 10° and 20°C. In these conditions it is important to maintain a proper soil moisture.

Recommendations for the nursery practice: Fruits of hornbeam should be collected when fully ripe and then dried at room temperature or lower and dewinged. Partially dried nutlets can be stored in tightly sealed bottles at a temperature of 3°C or lower until stratification time. Stratification should begin about 105–120 days before the expected date of spring sowing regardless of whether the sowing is to take place in the first season after collection or in the second. Stratification should run first for 14, 21 or 28 days at 20°C and then for no less than 90 days at 5°C. (Warm-followed-by-cold stratification). On observing the first germinating seeds the nutlets should be immediately sown to a depth of 1 cm. The optimal temperature of the soil or other medium for the energetic emergence of seed is 20°C. Under field conditions the seedlings will begin to emerge in about 15 days.

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#### SUMMARY

In the period 1963–1966 investigations were undertaken in the Department of Dendrology and the Kórnik Arboretum of the Polish Academy of Sciences at Kórnik near Poznań in order to establish the conditions of breaking dormancy and germination of seeds of the hornbeam (*Carpinus betulus* L.). Seeds from seven trees growing in the Kórnik Arboretum were used for the studies.

The nutlets were collected at full ripeness, always in November. They were dried after harvesting at room temperature. The water content was determined after drying for 48 hrs at  $105^{\circ}$ C and recalculated to percent by weight of fresh weight of the nutlets. Seed viability was determined by the cutting test. After drying and removing the wings, the seeds were stored in tightly closed jars at  $3^{\circ}$ C.

For stratification, a mixture of damp sand and peat (1:1 by vol.) was used. Germination was checked during control observations performed in the course of stratification at low temperatures  $(1^{\circ}-10^{\circ})$  every 3 weeks in germination tests, and at  $20^{\circ}$  at 6-day intervals. Seedling emergence tests were done in laboratory conditions in dark phytotron chambers at various temperatures ranging from 5° to  $20^{\circ}$ C. For this purpose the nutlets were sown in plastic boxes with a damp sand-peat mixture in 1-cm deep holes made with a templet. Then the holes were filled with dry sand immediately damped. The germination was checked at 3-day intervals, and all seeds the cotyledones of which had perforated the covering sand layer were removed each time. Simultaneously with the laboratory tests, germination of seeds sown in the ground at a depth of 1 cm was observed. All studies on stratification, germination and sprouting were made in four replications with 50 seeds each time.

Temperature variations in laboratory experiments did not exceed  $\pm 0.5^{\circ}$ C.

The following conclusions were reached:

(1) The hornbeam nutlets should be harvested at full ripeness. Earlier seed collection is of no use.

(2) Drying of the nutlets up to 9-11 percent of water content in fresh mass immediately after the harvest at room or lower temperature has no detrimental effect on the viability and germination of the seeds. It makes no difference whether stratification of the dried nutlets is started immediately after drying or after a year's storage in closed containers at low temperature (3° or less).

(3) Freshly collected nutlets  $(17-19^{0}/_{0}$  water content) or those dried after harvest  $(9-11^{0}/_{0})$  water) stratified immediately after being collected or after drying, eventually after drying and a years's storage, germinate in highest percent after warm-cool stratification. This should include a 2-4-weak warm phase at 20°C, and a following cool phase at 5°C. Beginning of germination under such conditions falls to the 12-14th week of the cool phase. The period between germination of the first and the last seeds for most trees lasts about 9 weeks (at 5°C).

(4) Stratification exclusively at temperatures of  $1^{\circ}$ ,  $3^{\circ}$  and  $5^{\circ}C$  is but little effective as regards the hornbeam. At  $10^{\circ}C$ , however, the seeds of some trees germinate in a high percent. The course of germination at  $10^{\circ}C$  is slow and its beginning is delayed.

(5) Temperatures of 1°, 3°, 5° and 10°C applied in the cool phase of warm-cold stratification were equally and highly effective as regards germination of seeds of most trees. The seeds germinate earliest and most intensively at 5°C, and latest at 10°C.

(6) Prolongation of the warm phase in warm-cool treatment to 12 weeks (the longest period tested) does not lower the viability and germination of the seeds.

(7) Hornbeam seeds germinate most vigorously when the temperature of the cool phase is raised to  $20^{\circ}$ C after 12–14 weeks of stratification. At this temperature all partially germinated seeds, those from cracked nutlets and most of the healthy intact seeds germinate for 6–8 days. Application of temperatures lower than  $20^{\circ}$ C delays the beginning of germination and lowers its rate. As the cool phase is shortened, the germination ability of the seeds to germinate at higher temperatures decreases. Protraction of the cool phase beyond 12–14 weeks causes germination of a larger number of seeds before temperature is raised, thus an undesirable phenomenon.

(8) Most favorable for seeding at raised temperature is the period of appearance of germinating seeds still in the cool phase of stratification. In optimal conditions of warm-cool stratification ( $20^{\circ}C/5^{\circ}C$ ) germination occurs after 12–14 weeks of cool treatment. Almost all seeds are then ready for germination, and raising of temperature does not allow the seeds to return to a state of secondary rest.

(9) Warm-cool stratification of hornbeam seeds ensures the highest yield of seedlings also in field conditions if the seeds are sown in a period when mean 24-hr soil temperature at seeding depth (1 cm) varies between 10°C and 20°C. An important factor in this case is the maintenance of adequate soil moisture.

Indications for nursery practice: Hornbeam nutlets should be collected in a state of full ripeness, dried at room or lower temperature, and the wings removed. After drying the nutlets should be stored in tight jars at  $3^{\circ}$ C or lower temperatures until stratification. This treatment should be started about 105–120 days before spring seeding, notwithstanding whether the seeds will be planted on the first or second spring after the harvest. The seeds should first be stratified for 14, 21 or 28 at 20°C, and further for not less than 90 days and 5°C (warm-cool treatment). When in the first lot of stratified seeds germination is noted, the nutlets should immediately be sown at a depth of 1 cm. Most favorable for vigorous germination is a soil (or other substrate) temperature close to 20°C. In conditions of field seeding germination starts after about 15 days.