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Bolesław Suszka and Kazimierz Krawiarz

Preparation of non stored red oak seed (*Quercus borealis* Michx.) for germination

INTRODUCTION

The North American red oak (*Quercus borealis* Michx.) produces seeds, the dormancy of which can be broken in conditions of cold stratification. According to the Woody-Plant Seed Manual (1948), this stratification should be conducted at a temperature of 0° - 3.3°C for 30 - 45 days, while germination tests should be conducted at an alternating temperature within the range 20° - 30°C. Brown (1939) who has studied the germination of red oak seeds stratified at various temperatures ranging from 1° to 20°C has concluded that optimum temperatures for this species are 10°C and 12.5°C. According to Heit (1942) the best germination results in the field conditions following a spring sowing are obtained when the seeds are first stratified for 75 - 90 days at 4°C.

The resistance of not partially dried and not stratified acorns of red oak to low temperatures is considerable. According to Zahariev and Conev (1958) and Velkov (1959) acorns of this species, containing 45 - 55% of water in the fresh weight, have resisted without loss of viability and without any injury, temperatures below 0°C reaching as low as —8°C. From —10°C onwards damage to some parts of the embryo or the freezing of the whole acorns has been noted.

Sanesi (1960), who has developed for several oak species a quick method of testing germination, suggests that acorns of red oak should be cut down by about 1/3 of their size, the hard external cover (pericarp) should be removed, and the seeds soaked in water for 48 hours, after which the seed germination test should be conducted in sand, for 28 days at a temperature of 20° - 30°C. Bonner (1968) has demonstrated an acceleration and facilitation of water uptake by previously stratified seeds of red oak that have been deprived of the pericarps, as well as by those on which the pericarp was split as compared to acorns with undamaged pericarps. According to this author the thin layer of wax covering the surface of the acorn represents a certain barrier to the absorption of

water. Some of the water is being absorbed by the readily permeable cup scar.

The breaking of dormancy in the seeds of red oak under controlled temperature conditions, the effect of their soaking on germination, the absorption of water, as well as the possible presence of growth regulators in the acorns, were the subject of the present investigation.

MATERIALS AND METHODS

Freshly fallen and not stored acorns from two trees of red oak growing in the Kórnik Arboretum have been collected in the autumn of 1968.

The viability of seeds has been estimated by the cutting test. The water content was expressed as percentage of the fresh weight lost after drying of the cut acorns at a temperature of 105°C for 48 hours.

For the stratification a moist mixture of sand with rubbed peat (1 : 1 vol.) was used. The same medium was used for the sowing of the acorns under controlled conditions. Temperature fluctuations in the experiments did not exceed ±0.5°C. In the experiments on stratification 3 or 4 replications with 50 seeds each were used.

In the studies on the breaking of dormancy during stratification we have followed the course of pericarp splitting, the emergence of roots (minimum length 5 mm) and the emergence of the epicotyl from between the cotyledons. The observations have been conducted at short identical time intervals, namely every 7 days, for the temperature ranges of 1° to 10°C and every 5 days for those at 20°C. Decayed seeds have been rejected during each observation period.

A SHORT CHARACTERISTIC OF THE EXPERIMENTS

Experiment 1

Purpose: To determine the optimal constant cold stratification temperature for whole acorns.

Design: The acorns were stratified at constant temperatures of 1°, 3°, 5° and 10°C.

Acorns collected: 12. 10. 1968, tree no. 1.

Experiment begun: 12. 10. 1968.

Initial seed viability: 99.5%.

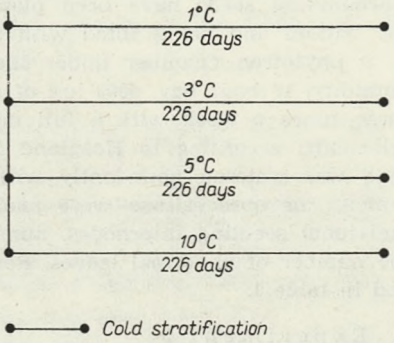
Initial water content of the whole acorns: 51.0% of the fresh weight.

Scheme: fig. 1.

Replicates: in each experimental variant 3×50 seeds.

Results: fig. 7.

Fig. 1. Scheme of experiment 1



Experiment 2

Purpose: To determine the optimal duration of cold stratification before testing germination at an elevated temperature.

Design: The acorns were stratified for 0, 14, 28, 42, 56 and 70 days at a temperature of 3°C, after which the stratification temperature was raised to 20°C for a period of 100 days.

Acorns collected: 28. 10. 1968, tree no. 1.

Experiment begun: 30. 10. 1968.

Initial seed viability: 99.0%.

Initial water content of the whole acorns: 48.3% of the fresh weight.

Scheme: fig. 2.

Replicates: in each experimental variant 4×50 seeds.

Results: fig. 8.

N. B.: Germinating seeds obtained in individual variants of the experiment have been used for the study of seedling growth under controlled conditions.

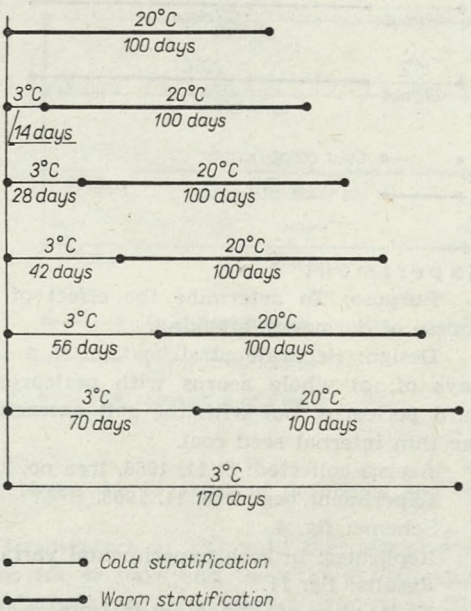


Fig. 2. Scheme of experiment 2

Germinating seeds have been planted singly in plastic boxes having a hole at the bottom and being filled with a sterile sand. The experiment was conducted in a phytotron chamber under the following conditions: 20°C, 80% relative air humidity, 16 hour day, 4000 lux of illumination. The sand was abundantly watered three times a week with a full nutrient medium (containing micro- and macro-elements) according to Hoagland (W e n t 1957), and on the remaining days the sand was watered abundantly with distilled water. At 10 day intervals measurements or observations were made of the following characters: length of the individual seedling internodes, number of internodes, total number of leaves and the number of abnormal leaves. Results of this experiment are presented in fig. 9 and in table 1.

E x p e r i m e n t 3

Purpose: To determine the optimal temperature for cold stratification preceding the raising of stratification temperature for the germination test.

Design: 49-day cold stratification at temperatures 1°, 3°, 5°, and 10°C after which the temperature was raised to 20°C for 177 days.

Acorns collected: 12. 10. 1968, tree no. 1.

Experiment begun: 12. 10. 1968.

Initial seed viability: 99.5%.

Initial water content of the whole acorns: 51.0% of the fresh weight.

Scheme: fig. 3.

Replicates: in each experimental variant 3×50 seeds.

Results: fig. 10.

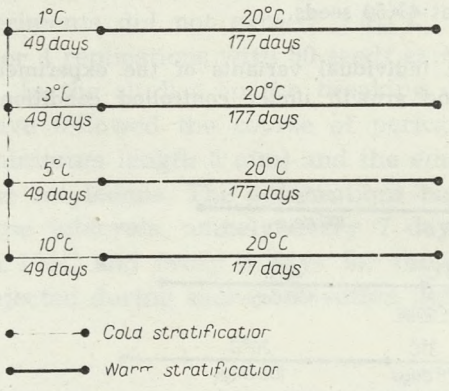


Fig. 3. Scheme of experiment 3

E x p e r i m e n t 4

Purpose: To determine the effect of seed covers (pericarp and testa) on the course of dormancy breaking.

Design: Separate stratification at a temperature of 3°C and at 20°C for 200 days of: a) whole acorns with pericarps undamaged; b) seeds deprived of the hard pericarps, but with the soft external testa left intact; c) embryos with only the thin internal seed coat.

Acorns collected: 8. 11. 1968, tree no. 2.

Experiment begun: 8. 11. 1968.

Scheme: fig. 4.

Replicates: in each experimental variant 2×25 seeds.

Results: fig. 11.

N. B.: A part of the seeds from series a), b) and c) have been used for stu-

dies on the occurence of growth regulators (inhibitors). More information on the subject can be found in the chapter on „results". Results of the studies on the occurence of growth inhibitors are presented in fig. 12.

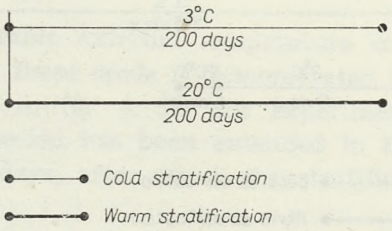


Fig. 4. Scheme of experiment 4

Experiment 5

Purpose: To study the effect of 1-week soaking of the acorns prior to stratification on the breaking of dormancy during stratification and on the germination of seeds.

Design: The acorns have been soaked for one week in water at a temperature of 3°C, with the water being changed daily, and then they were stratified at a temperature of 3°C for 225 days or for only 49 days after which the stratification temperature was raised to 20°C. Control variants without the soaking have also been included.

Acorns collected: 12. 10. 1968, tree no. 1.

Experiment begun: 12. 10. 1968.

Initial seed viability: 99.5%.

Initial water content in the whole acorns: 48.5% of the fresh weight.

Scheme: fig. 5.

Replicates: in each experimental variant 3×50 seeds.

Results: fig. 13.

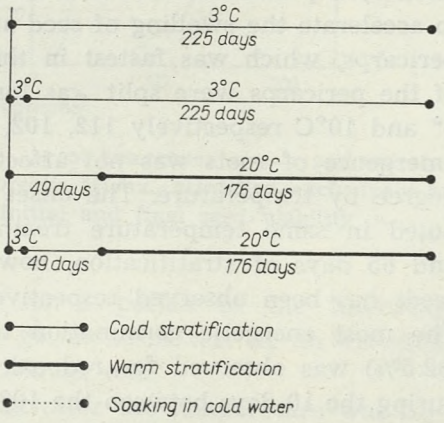


Fig. 5. Scheme of experiment 5

Experiment 6

Purpose: To determine the relation between the breaking of dormancy and the content of water in the seeds.

Design: Acorns were stratified at a temperature of 3°C until they germinated (115 days), or else in the same conditions for 49 days and then the stratification temperature was raised to 20°C and held there until the seeds germinated. The

water content was measured at weekly intervals, except in the first week when it was done daily and except the first day when it was done after 0, 6, 12 and 24 hours.

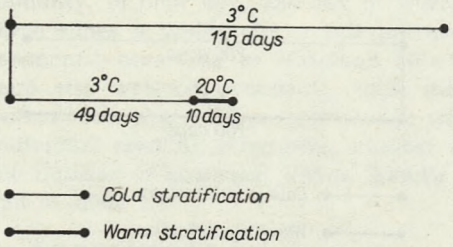


Fig. 6. Scheme of experiment 6

Acorns collected: 28. 10. 1968, tree no. 1.
Experiment begun: 30. 10. 1968.
Initial seed viability: 99.0%.
Initial water content in the whole acorns: 48.3% of the fresh weight.
Scheme: fig. 6.
Replicates: in each variant for the water content estimation 3×20 acorns.
Results: fig. 14.

RESULTS

THE BREAKING OF DORMANCY AND SEED GERMINATION

In figure 7 the course of dormancy breaking in the seeds of red oak during stratification at various constant temperatures (1° - 10°C, experiment 1) is presented. An increase in the stratification temperature seems to accelerate the swelling of seed and as a result also the splitting of the pericarps, which was fastest in the 10°C variant. The time when 80% of the pericarps were split was for the variants at temperatures 1°, 3°, 5° and 10°C respectively 112, 102, 94 and 48 days of stratification. The emergence of roots was not affected in the same way or to the same degree by temperature. The onset of germination (root emergence) was noted in same temperature treatments respectively after 124, 104, 92 and 65 days of stratification, however the germination of 80% of the seeds has been observed respectively after 162, 140, 123 and 160 days. The most energetic germination that reached the highest percentage (92.5%) was observed for red oak seeds stratified at 5°C. In this case during the 10 days between the 100th and 110th day of stratification 65% of the seeds germinated. Of special note is the fact that during stratification at temperatures of 1° - 5°C growth of epicotyls was never recorded. Thus, these temperatures efficiently inhibit the elengation of this part of the embryo. The emergence of the epicotyls has been observed only after germination at 10°C, that is in thermal conditions in which the rate of germination is slower than at 3° or 5°C. The causes of inhibition

of the first phase of epicotyl growth lie exclusively in the external conditions, since the transfer of acorns form any of these temperatures to 20°C (see exp. 3) has led immediately to an elongation of the epicotyl in all the healthy seeds.

The true, not inhibited by unfavourable external temperature conditions, course of dormancy breaking in these seeds is demonstrated by the results of experiment 2, presented in fig. 8. In this experiment, the duration of the cold stratification period has been extended in the successive experimental variants by 14 days, after which the stratifica-

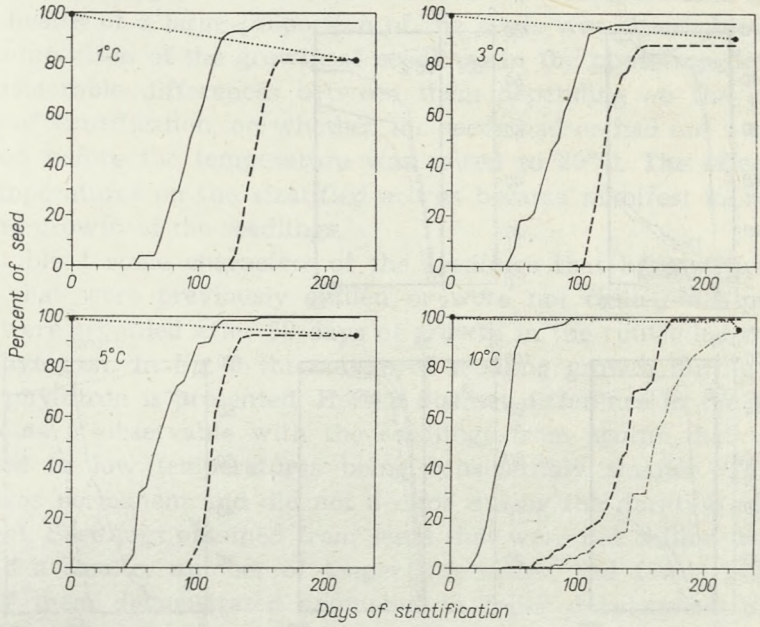


Fig. 7. Experiment 1. Pericarp splitting ——— , root emergence ------ and epicotyl emergence ——— in acorns of *Quercus borealis* Michx., stratified exclusively in the cold condition at 1°, 3°, 5° and 10°C. Initial and final seed viability •.....•

tion temperature was raised to 20°C for a period of the following 100 days, which was considered as a germination test at an elevated temperature following stratification at a lower one.

In experiment 2 it was observed that after the temperature was raised the pericarps soon split in all the sound acorns, while germination (root emergence) and the elongation of epicotyls which quickly follows it were dependent on the preceding duration of the cold stratification. First to germinate were the seeds which had behind them 56 and 70 days of initial cold stratification. After 70 days of the cold stratification and 10 days at 20°C 80% of the acorns germinated while after a further

10 days in the warm conditions already 98% of the seeds, that is all the healthy ones were germinated. Already 4 days later an active elongation of all the epicotyls was observed. Thus in comparison with the constant 3°C control variant in this experiment the elevation of the temperature from 3°C to 20°C after 70 days of cold stratification has resulted in a marked acceleration of seed germination. Germination of 80% of the seeds was observed here not after 124 days as in the 3°C control but after 80 days, counting from the day on which stratification was begun, that is 44 days earlier. It is also necessary to point out that during cold stratification the growth of the epicotyls was not observed at all.

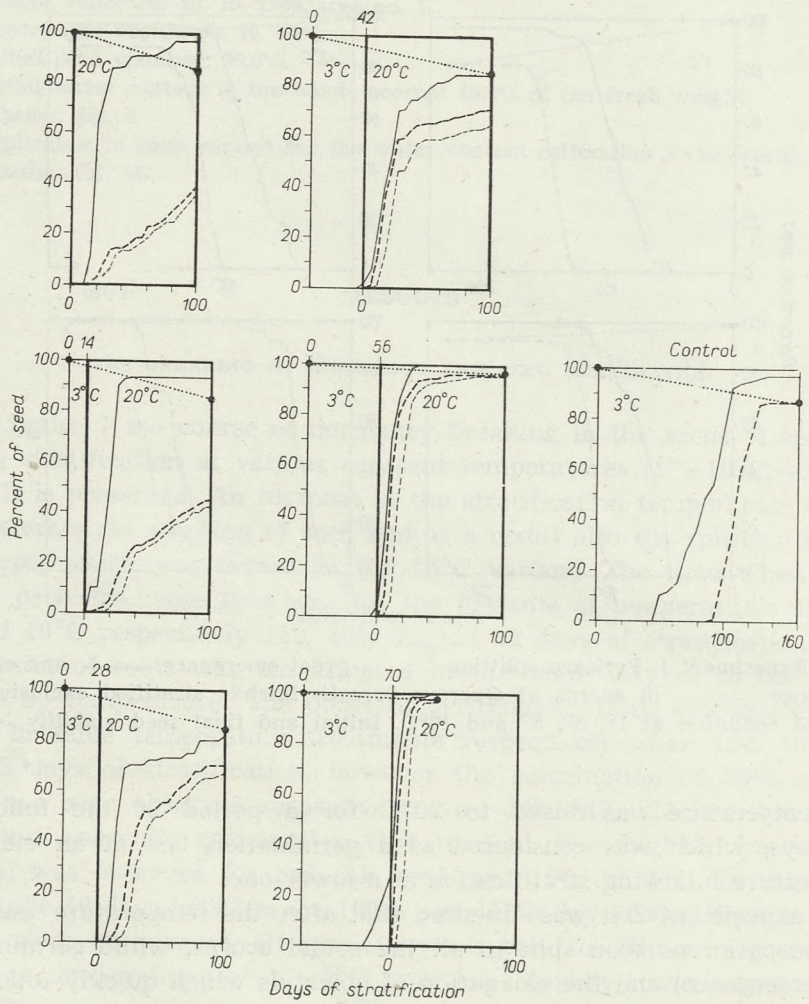


Fig. 8. Experiment 2. Pericarp splitting ——— , root emergence – – – – – , and epicotyl emergence ····· in acorns of *Quercus borealis* Michx., stratified in the cold-followed-by-warm regime for 0, 14, 28, 42, 56 or 70 days at 3°C and then for 100 days at 20°C. Initial and final seed viability .·····.

It is also worth mentioning that in this experiment, the acorns which were kept at a constant temperature of 20°C without previous stratification at a cold temperature have slowly but continuously germinated reaching eventually a value of 40% seeds with emerged roots and 36% with elongated epicotyls. The course of the germination curves indicates that in this case the percentage of germinated seeds would be even greater if we were to extend the germination time beyond the studied 100 days. It has to be pointed out that the elongation of the cold stratification beyond the initial 42 days has resulted in a considerable improvement in the viability of the seeds at the time of experiment termination. Without a sufficiently long period of the action of low temperatures a decline in the health of a large proportion of the seeds was observable.

A comparison of the growth of seedlings in the phytotron demonstrates considerable differences between them depending on the preceding regime of stratification, on whether the seeds had or had not a cold stratification before the temperature was raised to 20°C. The effect of the low temperatures on the stratified acorns became manifest in the structure and growth of the seedlings.

In table 1 some characters of the seedlings that have grown out of acorns that were previously chilled or were not chilled are presented. These were recorded after 60 days of growth in the controlled conditions of a phytotron. In fig. 9 the course of seedling growth during 90 days in the phytotron is presented. Here a distinct difference in the length of the shoots is observable with the seedlings from acorns that were not stratified in low temperatures being considerably smaller. This difference was permanent and did not decline during the duration of the experiment. Seedlings obtained from seeds that were not chilled were shorter, had a smaller number of longer internodes, had fewer leaves, and most of them demonstrated anomalies in foliar development. Seeds of red oak are therefore capable of slow germination in conditions of raised temperature, without the need for prior cold stratification, however the appearance of the seedlings obtained from such seeds indicates that the dormancy of the epicotyl has not been totally overcome. On the basis of this information it is possible to include the seeds of red oak in the group of seeds characterized by weak dormancy.

On the basis of the experiments conducted it turned out that for the red oak that has become acclimatized in Poland the optimal duration of cold stratification is 70 days at 3°C, after which when the temperature is raised to 20°C there results a rapid, copious and energetic germination (root emergence) and epicotyl elongation of all the healthy seeds with a minimal decline in viability. The raising of the temperature also does not induce any secondary dormancy which for seeds of other speces under similar conditions has been shown to happen by Nikolaeva (1964) and Suszka (1967).

In experiment 3 a somewhat longer cold stratification period was employed than suggested by the Woody-Plant Seed Manual (1948), namely of 49 day duration before the temperature was raised. Results of this experiment (fig. 10) confirmed those presented above. Of the temperatures used for the cold stratification (1°, 3°, 5° and 10°C) the 5°C temperature proved most satisfactory since after it the germination of the roots and the elongation of the epicotlys had the most energetic

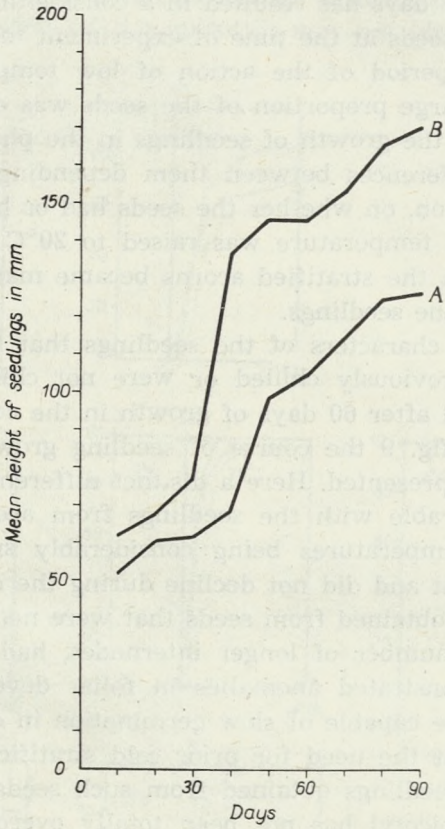


Fig. 9. Experiment 2. The course of seedling growth of *Quercus borealis* Michx., from acorns that have germinated during exclusively warm stratification at 20°C (*A*), or during a warm stratification at 20°C that was proceded by 70 days of a cold stratification at 3°C (*B*)

course, together with the percentage of germinated seeds being highest. The germination of roots and the growth of the epicotyl of seeds earlier stratified at 1° and 3°C has taken place in the raised temperature but considerably delayed compared with the result following the optimal 5°C stratification.

The 49-day period of cold stratification proved here also to be too

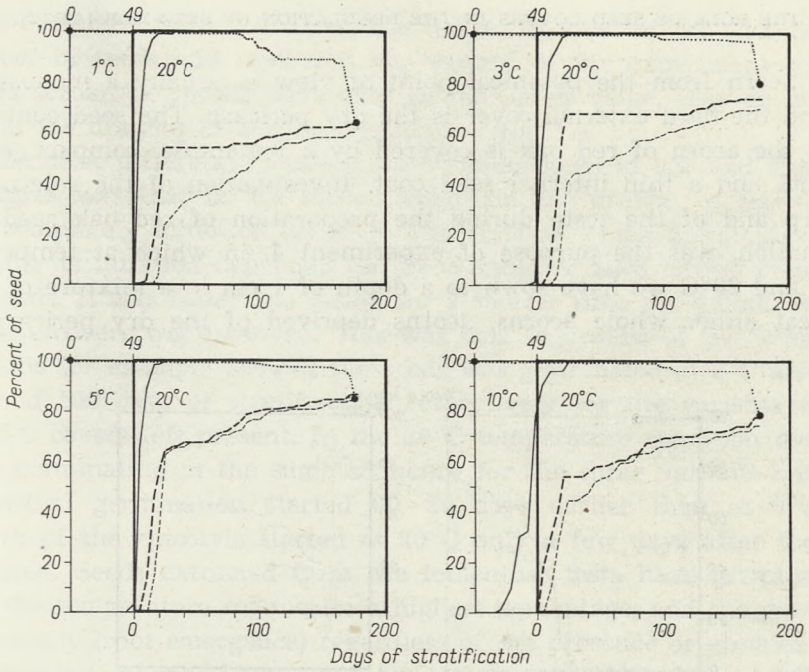


Fig. 10. Experiment 3. Pericarp splitting ——— , root emergence – – – – and epicotyl emergence ——— in acorns of *Quercus borealis* Michx., stratified in the cold-followed-by-warm regime at 1°, 3°, 5° and 10°C followed by 20°C. Seed viability .....

short for the complete breaking of dormancy in all the roots and epicotyls. The results obtained have therefore an intermediate position in relation to the results of variants in experiment 2 with a 42-day and a 56-day cold stratification periods. Thus the suggestion that the duration of the cold stratification time be extended finds an indirect confirmation also in the results of experiment 3 discussed here.

Table 1

Some seedling characters of red oak (*Quercus borealis* Michx.) after 60 days of growth in a phytotron / 20°C, 80% relative air humidity, 16 hour day, illumination 4000 lux, sand cultures watered 3 times a week with a nutrient medium containing micro- and macro-elements (after Hoagland)

	Seedlings from seeds germinated at 20°C without prior cold stratification	Seedlings from seeds germinated at 20°C following a 70 day stratification at 3°C
Mean shoot length in mm	105.0	144.4
Mean no. of internodes	2.6	6.2
Mean internode length in mm	41.8	25.6
Mean no. of leaves	6.2	8.6
Percentage of seedlings with abnormal leaves	60.0	0

THE ROLE OF SEED COVERS IN THE REGULATION OF SEED DORMANCY

An acorn from the botanical point of view is usually a monoseeded nut, and the hard external cover is the dry pericarp. The seed contained within the acorn of red oak is covered by a tomentose, compact, external testa and a thin internal seed coat. Investigation of the role of the pericarp and of the testa during the preparation of red oak seeds for germination, was the purpose of experiment 4, in which at temperatures 3° and 20°C we have sown to a depth of 1 cm in a mixture of sand and peat either whole acorns, acorns deprived of the dry pericarps or

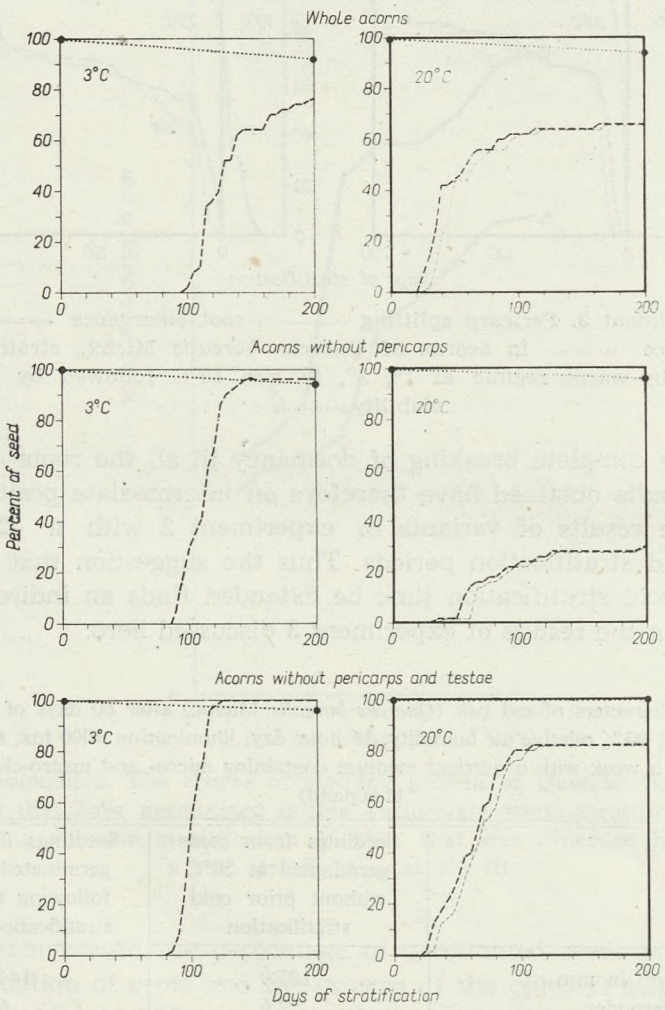


Fig. 11. Experiment 4. The emergence of roots ------ and epicotyls ——— from acorns of *Quercus borealis* Michx., stratified at 3° or 20°C. Initial and final seed viability .....•..... . Whole acorns, acorns without the pericarps and acorns without the pericarps and without the external tomentose testae have been stratified

seeds extracted from the tomentose testae. In view of the fact that the number of seeds and replicates per variant of the experiment was low (2×25 seeds) the results have only an indicatory value, they do however permit the drawing of certain conclusions (fig. 11).

The basic difference between the variants sown in low and high temperatures was that in the former condition the growth of the epicotyl was completely inhibited (at 3°C). The time at which germination started and its duration depended on the number of seed covers present on the seeds. It was faster and lasted for a shorter time the greater number of these covers was removed. This was true regardless of the temperature. Thus for example 50% of the seeds was germinated at 3°C after 130, 112 and 100 days of stratification respectively for the variants with 3, 2 and 1 covers left present. In the 20°C temperature condition the seeds were germinating in the same sequence for the three variants only that the actual germination started 60 - 80 days earlier than at 3°C. The growth of the epicotyls started at 20°C only a few days after the roots appeared. Seeds extracted from the tomentose testa have germinated in both the temperature regimes to a highest percentage, and the germinative capacity (root emergence) regardless of the presence or absence of the pericarp was always lower at a temperature of 20°C than at 3°C. The surprisingly low germinative capacity of seeds deprived only of the pericarp and sown at 20°C could have been accidental. So far it was not possible to find any reasons for that. The acceleration and enhancement of the germinative capacity, obtained as a result of removing seed covers indicates that both the pericarp and the testa have an inhibiting effect on onset of root and epicotyl growth of the embryo. The reasons for this can be sought both in the presence of hypothetical growth inhibitors as well as in the restriction of water uptake and gaseous exchange between the external environment of the acorns and the embryo. An attempt to obtain answers to some of these questions is described in the experiments discussed below.

THE PRESENCE OF GROWTH REGULATORS IN THE ACORNS

In order to obtain information about the possible occurence of growth regulators in mature not partially dried and not stratified acorns of red oak, we have extracted with water at a temperature of 3°C for 36 hours 15 acorns in each of the following variants:

a) whole acorns with the pericarps undamaged

b) whole seeds with the pericarps removed but with the tomentosa testae intact

c) whole embryos extracted from the testae but with the membraneous internal seed coats left intact

d) homogenized embryos with the internal membraneous seed coats.

The water extracts after centrifugation and filtration have been acidified and treated by the method described by Krawiarz (1970). The preliminarily purified acid ether fractions have been studied for the content of growth regulators with the help of a biotest (wheat coleoptile), following paper chromatography of the extracts in the system isopropanol-ammonia-water (10 : 1 : 1). Results of the biotests are presented in fig. 12.

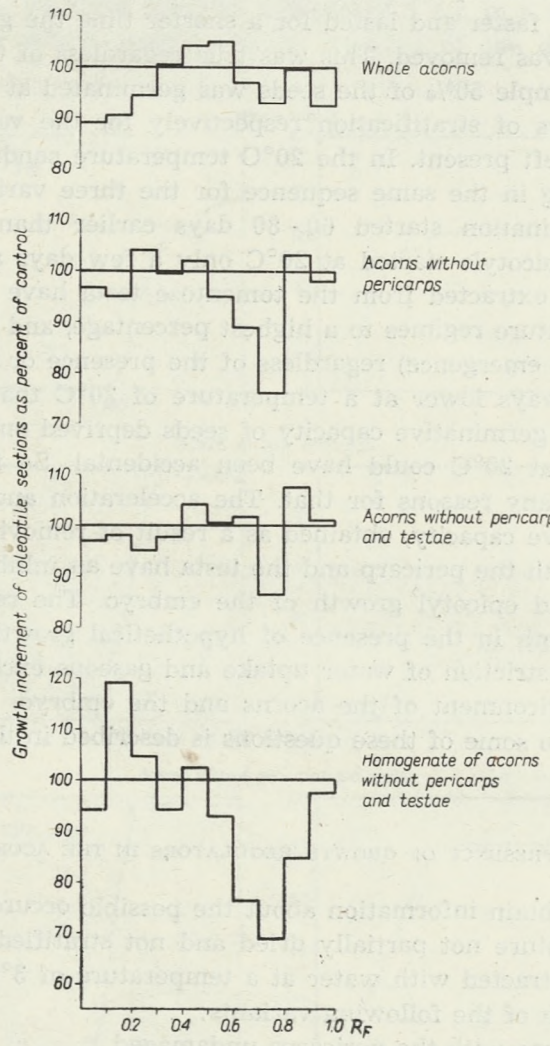


Fig. 12. Experiment 4. Results of biotests (wheat coleoptile elongation test) following chromatography in the isopropanol-ammonia-water (10 : 1 : 1) solvent of the acidic ether fraction of cold water extracts from acorns of *Quercus borealis* Michx.: from whole acorns, from acorns with the pericarps removed, from acorns with the pericarps and the tomentose external testae removed and from homogenates of acorns deprived of the pericarps and of the external testae

In all the extracts the presence of a growth inhibitor was demonstrated at an R_F value of 0.7 - 0.8, that is in the same position as is taken during chromatography in the same system by abscisic acid, the well known growth inhibitor that has already been found in many higher plants. An especially high concentration of this inhibitor has been found in the extracts from seeds with the tomentose testae as well as from the homogenate of the embryos with the membraneous internal seed coats, but without the testae. It appears that each of the seed covers inhibits extraction from tissues present underneath it. In this experiment it was possible to demonstrate the presence of a relatively high concentration of an unidentified inhibitor in the tomentose external testae and in the embryos themselves of red oak. In view of the only tentative nature of these results we lack at the moment any basis to associate the presence of this inhibitor with the behaviour of the stratified seeds in experiment 4 in which also either whole acorns were used or acorns that have been peeled as in variants a), b) and c) of this experiment.

THE INFLUENCE OF SOAKING OF THE ACORNS ON THEIR GERMINATION

We have used for the acorns of red oak the treatment of soaking seeds in water sometimes recommended (P a l m e r 1955, J o n e s 1958, M e s s e r 1960), for other oak species. In our experiment we have used a cold (3°C) water changed daily. The acorns were soaked both before the continuous cold stratification of long duration and before the 49-day stratification in the same condition followed by the raising of the temperature to 20° (experiment 5). The results of this experiment presented in fig. 13 indicate that the soaking treatment before the exclusively cold stratification was ineffective. Such seeds have later germinated to a much lower percentage than the control seeds which were not soaked, and the viability of these seeds has substantially declined during the course of the stratification. Growth of the epicotyl has not been observed at a temperature of 3°C neither in the soaked nor in the control acorns. The soaked seeds which were transferred from the cold stratification to a higher temperature have germinated to about the same degree as the control seeds which were not soaked, but better than the soaked seeds which were held in the low temperature conditions only. The growth of the epicotyl started almost immediately after the appearance of the radicle. It has to be pointed out, that in all the variants of the experiment, the pericarps have split in almost all the sound seeds, quite apart from the established differences in the germinative capacity. Similarily as in the other experiments the splitting of the pericarps was significantly accelerated by the raising of the stratification temperature. The injurious effect of soaking acorns in a daily changed but standing water, that has been

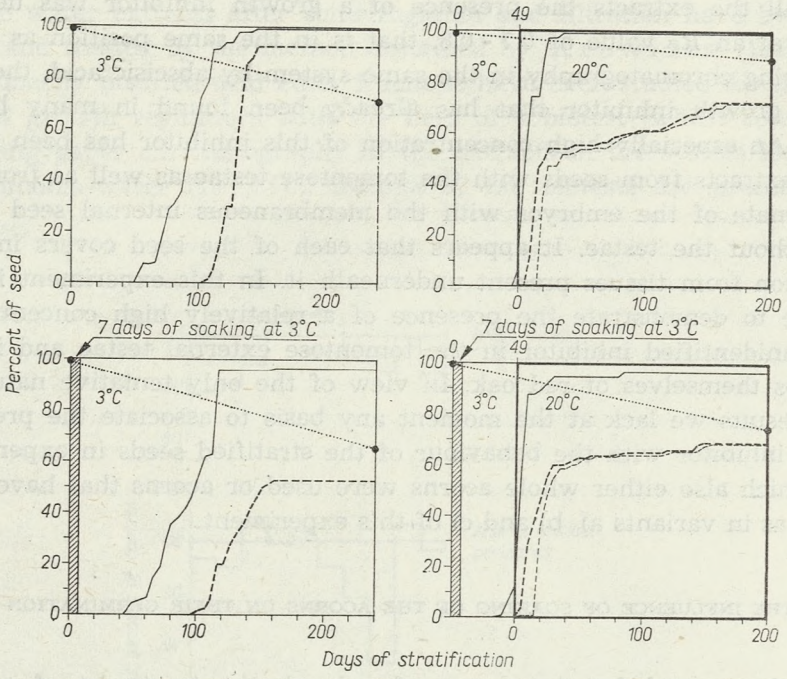


Fig. 13. Experiment 5. Pericarp splitting _____, the emergence of roots _____ and emergence of epicotyls ..... in acorns of *Quercus borealis* Michx. during exclusively cold stratification at 3°C and cold-followed-by-warm stratification (49 days at 3°C and then at 20°C). The seeds were either not soaked or soaked for 7 days in distilled water changed daily and maintained at 3°C. Initial and final seed viability .....

demonstrated here, could have been caused by a restriction of gasous exchange in the acorns that have imbibed water, and particularily the uptake of oxygen from water may have been limited.

WATER CONTENT IN THE ACORNS

In experiment 6 we have followed the changes in water content of the whole acorns from the day the experiment was begun until the acorns germinated, both in the conditions of gradual preparation for germination by cold stratification and in the conditions of the cold-followed-by-warm stratification (49-days at 3°C and then at 20°C). Determinations of the water content have been conducted until the 49th day of the cold stratification at weekly intervals except that in the first week the determinations were made every day and in the first day after 0, 6, 12 and 24 hours.

The results are presented in fig. 14. As can be seen the initially high water content of the freshly fallen acorns (48.3%) undergoes relatively

minor changes during cold, or cold-followed-by-warm stratification. In the first week of stratification it rose to 50,0%. Acorns with split pericarps contained after 49 days of cold stratification 54.6% of water and the acorns that were germinating had after 115 days 56.7% of water in the fresh weight. Acorns which on termination of the cold stratification in the 49th day and on being transferred to a temperature of 20°C have

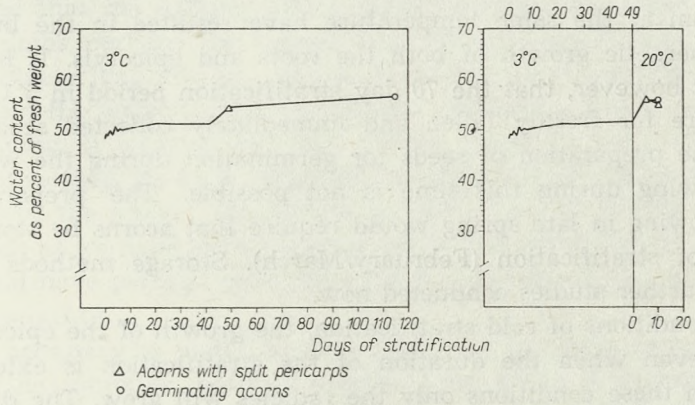


Fig. 14. Changes in the water content (as percentage of fresh weight) in the acorns of *Quercus borealis* Michx. during exclusively cold stratification (3°C) or cold-followed-by-warm stratification (49 days at 3°C and then at 20°C)

germinated energetically and quickly, contained after staying in the higher temperature for 10 days the following water contents; 55.3% for acorns that have not germinated but had split pericarps and 55.7 for the germinated seeds. The total increase in the water content in relation to the value present at the time of acorn collection was only 7.4%.

DISCUSSION

The experiments conducted indicate that the thermal conditions required for the breaking of dormancy of red oak acorns collected in Kórnik differ considerably from the requirements reported in the Woody-Plant Seed Manual (1948). The Manual has data for acorns collected within the natural range of the species, while our material was collected in Poland where red oak is very well acclimatized. For the studies conducted here only two seed lots have been used, it can however be pointed out that the data reported in the Manual are based on germination tests of *Quercus borealis* Michx. conducted on 11 samples and for the geographical variety *Q. borealis var. maxima* Ashe on only 3 samples, and thus also on very scanty material. Our results are closer to the data of Heit (1942) referred to at the beginning of this paper.

Acorns from the trees growing in the Kórnik Arboretum required a much longer stratification than the 30 - 45 days recommended by the Woody-Plant Seed Manual, namely 70 days and the best temperature conditions were 5°C and not 0° - 3.3°C as recommended. In our conditions it was at this temperature that the seeds of red oak were best prepared for germination. The raising of the temperature to 20°C after such a long period of cold stratification or the sowing out of the seeds into a substratum at the same temperature have resulted in the immediate onset of energetic growth of both the roots and epicotyls. It has to be pointed out however, that the 70 day stratification period in 5°C recommended here for freshly fallen and immediately collected seeds would result in the preparation of seeds for germination during the winter. In practice sowing during this time is not possible. The preparation of seeds for sowing in late spring would require that acorns be stored until the onset of stratification (February/March). Storage methods are the subject of further studies conducted now.

In the conditions of cold stratification, the growth of the epicotyl does not occur even when the duration of the stratification is extended to 225 days. In these conditions only the radicles will grow. The difference in the reaction to temperatures of the radicles and of the epicotyls in the acorns of red oak deserves comment. While the epicotyls will not grow at temperatures between 1° and 5°C, the growth of the roots is possible at any temperature within a wide range from 1° to 20°C. The effect of the thermal conditions is evidenced here primarily in that at lower temperatures within this range the onset of root germination comes later. Epicotyls, which begin growth in high temperatures that have not been preceded by a sufficiently long period of acorn stratification at low temperatures grow up into shoots that are in many respects abnormal. During stratification the onset of active epicotylar growth becomes possible only at temperatures higher than 5°C. In the range of temperatures tested in our experiments, namely 1°, 3°, 5° and 10°C the epicotyls grew only at 10°C. They grow therefore normally only at higher temperatures provided that previously the acorns have been subjected to the action of low temperatures while moist and aerated. Otherwise their growth is possible but is reduced and abnormal. The reason for such an adaptation is obvious. This permits the acorns to germinate in farourable natural conditions and become rooted already in late autumn, and to sustain the winter under leaves and snow in this state. The gradually worsening thermal conditions during autumn and early winter do not permit the growth of the epicotyl and the shoot. This protects the shoots against damage by low winter temperatures and at the same time permits their growth in the spring when the temperature of the air and of the soil improves, while at the same time the water requirement is satisfied by the root system which is already well established in the deeper levels of the soil.

Soaking of the acorns in conditions described in this paper has proved to be an unfavourable treatment affecting the viability and germination capacity of seeds. The references in the literature recommending the soaking of acorns concern other species of oak. For the soaking Jones (1958) and Palmer (1955) recommend the use of pure tap water or even distilled water but not river water. Messer (1960) suggests that the ability to absorb water increases with the temperature of the water and that the soaking period should be relatively short (12 hours). This explains perhaps the reason why soaking was unfavourable since an excessively long duration of the treatment was employed in our conditions and the water though periodically changed and cold was none the less standing. It appears that only flowing, aerated water with a higher temperature than we have employed could have had a positive effect on the state of the acorns. The growth inhibitors, activated during swelling, and which as we have shown are present in the acorns of the studied species, could have perhaps been more easily washed out by water at a higher temperature.

It is necessary to underline that this work has demonstrated the presence of an inhibitor, possibly abscisic acid, which is exceptionally rich in the tomentose testae and in the embryos themselves. It is not unlikely that it explains to a certain degree the phenomenon of dormancy in acorns of red oak. Thus it would be necessary to conduct further studies covering also other phases of the preparation of acorns for germination and also for comparative purposes studies on the presence of growth inhibitors in acorns of such oak species that do not enter a dormant condition at all. Essential for the question would also be studies on the presence in acorns of red oak of other growth regulators, that is the growth promoters.

It is not possible to consider dormancy of red oak seeds as a deep one. It was shown that the roots are capable of active growth in a wide range of temperatures, and the moment when germination begins is not dependent nor related in time to achievement by the epicotyls of a state of readiness for extension growth and normal development. The normal development of the shoot requires that it be preceded by a long (70 days in our conditions) period when it is subjected to the action of low temperatures.

Results of our experiments, in which whole acorns were stratified, as well as seeds deprived of the successive seed covers, permit us to conclude that the dormancy of red oak acorns may be associated both with the presence of the covers and with the physiological state of the meristematic tissues, particularily of the apical dome in the embryo, the meristematic activity of which can be biochemically blocked. Bonner (1968) has clearly shown that the pericarp of the acorns from this species is, as a result of being covered by thin layer of wax, to some extent

a barrier to the free entry of water to the seed over most of the acorn surface. The presence of a growth inhibitor, that has been demonstrated in this work, not only in embryo itself but also in the tomentose testa can point to another function of the seed covers than the prevention of water entry to the embryo.

The rate at which acorns swell depends according to Messer (1960) directly on the temperature. In the studies of Bonner (1968) on the acorns of red oak, they have quickly imbibed much water on being soaked in water at 19°C after stratification. In our experiments, the uptake of water during the cold and then during the warm stratification did not undergo major changes, in spite of the fact that the possibility existed in the moist stratification medium. This was also true for acorns with split pericarps or even germinating ones, both in cold stratification and during the warm treatment.

CONCLUSIONS

The results obtained permit the drawing of the following conclusions for undried and non stored acorns of *Quercus borealis* Michx. collected immediately after seed fall:

1) A cold stratification of red oak acorns at a temperature within the range 1° - 10°C is most satisfactory when at 5°C. The onset and termination of germination under conditions of this temperature falls on the 92nd and 144th day of stratification respectively. In the temperatures within the range 1° - 5°C the epicotyls do not elongate.

2) A cold-followed-by-warm stratification, during which acorns have been initially stratified at a temperature of 3°C for 70 days and later at a temperature of 20°C permits a very energetic germination and growth of the epicotyls in all the healthy acorns almost immediately after the appearance of the radicles. The onset and termination of germination falls in these conditions on the 70th and 90th day of stratification respectively (70 days at 3°C+20 days at 20°C).

3) Of all the temperatures used in this study for the cold period of stratification, preceding the warm stratification or sowing at 20°C, the most satisfactory one was 5°C. This temperature maintains the highest seed viability and in it all healthy acorns germinate.

4) Acorns of red oak are capable of germination in a moist medium at a temperature of 20°C without previous cold stratification. Germination under these conditions is less energetic and slower than at temperatures in the 1° - 5°C range, it begins however 60 - 80 days earlier and not only roots grow but also the epicotyls. Removal of the pericarps or both the pericarps and the tomentose testae does not accelerate germi-

nation at 20°C. The seedlings obtained from not after-ripened acorns have characters of physiological dwarfness in comparison with seedlings from acorns that have been stratified at low temperatures for 49 days before being transferred to higher temperatures.

5) The removal from the acorns of the pericarps and of the tomentose testae results in an increase of the percentage of seeds germinating at the low temperature (3°C) as well as at the higher temperature (20°C). At 3°C the removal of the pericarps and of the tomentose testae accelerates germination respectively by 20 and 30 days.

6) Soaking of seeds before stratification for 7 days in a daily changed distilled water at 3°C is an ineffective treatment in that it lowers the viability and germinative capacity of the seeds.

7) Regardless of the stratification method employed (cold or cold-followed-by-warm) the water content of the stratified acorns, which is already high initially (48% of the fresh weight) is only slightly increased in the course of stratification and during germination (7 - 8% in the germinating seeds above the initial value).

8) In non dried and not stratified acorns of red oak it was possible to demonstrate in the tomentose external testae as well as in the peeled acorns themselves a high concentration of an unidentified growth inhibitor, which was possibly abscisic acid.

Institute of Dendrology and Kórnik Arboretum
Kórnik nr. Poznań

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BOLESŁAW SUSZKA I KAZIMIERZ KRAWIARZ

Przysposabianie nie przechowywanych żołędzi dębu czerwonego (Quercus borealis Michx.) do kiełkowania

Streszczenie

W sezonie 1968/69 przeprowadzono w Zakładzie Dendrologii Polskiej Akademii Nauk w Kórniku koło Poznania badania nad ustępowaniem spoczynku nasion dębu czerwonego (*Quercus borealis* Michx.). Do badań użyto żołędzi nie podsuszonych, zebranych jesienią 1968 r., natychmiast po opadnięciu z drzew rosnących w Arboretum Kórnickim. Żołędzie stratyfikowano w wilgotnej mieszaninie piasku z drobno przetartym torfem (1 : 1 obj.). Okresowe kontrolne pękania owocni, kiełkowania (korzenie) i pojawiania się epikotyli przeprowadzono w temperaturach obniżonych (1°, 3°, 5° i 10°C) w odstępach 7-dniowych, a w temperaturze 20°C — co 5 dni. Za skiełkowane uznawano żołędzie z widocznym korzeniem o długości co najmniej 5 mm, za początek wzrostu epikotyli natomiast — swobodne wysunięcie się rosnącego pędu spomiędzy liścieni. Badania nad wzrostem siewek przeprowadzano w fitotronie w następujących warunkach: 20°C, 80% wilgotności względnej powietrza, 16-godzinny dzień, 5000 lux, 3-krotne w ciągu tygodnia podlewanie kultur piaskowych pożywką Hoaglanda zawierającą mikro- i makroelementy, w pozostałe dni tygodnia piasek podlewano wodą destylowaną. Zawartość wody oznaczano w odniesieniu do świeżej masy (procent wagowy), po suszeniu pokrajanych żołędzi przez 48 godzin w 105°C. Badania nad zawartością wody w stratyfikowanych żołędziach przeprowadzano w wyżej wymienionym podłożu o zawartości 35 - 40% wody w świeżej masie. Oceny stanu zdrowotności przeprowadzano przy użyciu metody krojenia. Obecność inhibitora wzrostu w kwaśnej frakcji eterowej wodnych ekstraktów z żołędzi oznaczano przy pomocy testu wydłużeniowego koleoptyli pszenicy (Ostka Chłopicka), po bibułowej chromatografii (Wh 3) wstępnie oczyszczonych ekstraktów solwentem: izopropanol-amoniak-woda (10 : 1 : 1).

Uzyskane wyniki pozwalają na wyciągnięcie następujących wniosków, dotyczących nie podsuszonych i nie przechowywanych żołędzi dębu czerwonego, zebranych natychmiast po opadnięciu z drzew:

1) Chłodna stratyfikacja żołędzi dębu czerwonego w temperaturach zakresu 1° - 10°C przebiega najskuteczniej w 5°C. Początek i koniec kiełkowania przypada w tej temperaturze na 92 i 144 dzień stratyfikacji. W temperaturach zakresu 1° - 5°C nie wydłużają się epikotyle.

2) Stratyfikacja chłodno-ciepła, podczas której żołędzie stratyfikowano począt-

kowo w temperaturze 3°C przez 70 dni, a potem w temperaturze 20°C, umożliwia bardzo energiczne skiełkowanie i wzrost epikotyli wszystkich zdrowych żołędzi, następujący prawie natychmiast po pojawieniu się korzeni. Początek i koniec kiełkowania przypada w tych warunkach na 70 i 90 dzień stratyfikacji (70 dni 3°C+20 dni 20°C).

3) Spośród wszystkich zastosowanych w tej pracy temperatur chłodnego okresu stratyfikacji, poprzedzającej stratyfikację lub wysiew w temperaturze 20°C, najbardziej skuteczną okazała się temperatura 5°C. Zapewnia ona najwyższą żywotność nasion, a wszystkie zdrowe żołędzie kiełkują.

4) Żołędzie dębu czerwonego są zdolne do kiełkowania w wilgotnym podłożu w temperaturze 20°C bez uprzedniej chłodnej stratyfikacji. Kiełkowanie przebiega w tych warunkach mniej energicznie i wolniej niż w temperaturach zakresu 1° - 5°C, rozpoczyna się jednak o 60 - 80 dni wcześniej, rosną nie tylko korzenie, ale i epikotyle. Żołędzie pozbawione owocni lub owocni i kutnerowatej zewnętrznej okrywy nasiennej kiełkują w temperaturze 20°C w tym samym terminie co żołędzie całe. Siewki uzyskane z niechłodzonych żołędzi wykazują cechy fizjologicznej karłowatości w porównaniu z siewkami z nasion stratyfikowanych przed podwyższeniem temperatury przez 49 dni w obniżonej temperaturze.

5) Zdjęcie owocni i kutnerowatej zewnętrznej okrywy nasiennej przyczynia się do podwyższenia procentu nasion kiełkujących zarówno podczas stratyfikacji w obniżonej (3°C), jak i podwyższonej temperaturze (20°C). W temperaturze 3°C zdjęcie owocni lub owocni wraz z zewnętrzną okrywą nasienną przyśpiesza kiełkowanie o odpowiednio 20 względnie 30 dni.

6) Moczenie żołędzi przed stratyfikacją przez 7 dni w codziennie zmienianej wodzie destylowanej o temperaturze 3°C, jest zabiegiem nieskutecznym i obniżającym żywotność i zdolność kiełkowania nasion.

7) Bez względu na zastosowany sposób stratyfikacji (chłodna lub chłodno-ciepła) zawartość wody w żołędziach zastratyfikowanych przy jej wysokiej zawartości początkowej (48% w świeżej masie) podlega podczas stratyfikacji i kiełkowania nieznacznej tylko podwyżce (o 7 - 8% w nasionach kiełkujących).

8) W nie podsuszonych po opadnięciu z drzew i nie stratyfikowanych jeszcze żołędziach dębu czerwonego stwierdzono w kutnerowatej, miękkiej zewnętrznej okrywie nasiennej i w pozbawionych jej nasionach wysoką koncentrację niezidentyfikowanego dotąd bliżej inhibitora wzrostu, być może kwasu abscysynowego.

БОЛЕСЛАВ СУШКА И КАЗИМЕЖ КРАВЯЖ

Приспосабливание нехраненных семян дуба северного (Quercus borealis Michx.) к прорастанию

Резюме

В 1968/69 гг. в Институте дендрологии Польской академии наук в Курнике изучался выход из состояния покоя семян *Quercus borealis* Michx. Для исследований были использованы желуди, собранные осенью 1968 г. сразу же после опадания их с деревьев, растущих в арборетуме. Желуди стратифицировались во влажной смеси песка с мелко протертым торфом (1 : 1 по объему). Периодические контрольные проверки растрескивания околоплодников, прорастания (корни) и появления эпикотилей проводились при низких температурах (1°, 3°,

5°, и 10°C) через семидневные промежутки времени, и при температуре 20°C — каждые 5 дней. Проросшими считались желуди с заметным корнем длиной не меньше 5 мм, началом же роста эпикотилей — свободное выдвижение растущего побега между семядолями.

Изучение роста сеянцев осуществлялось в фитотроне в следующих условиях: 20°C, 80% относительной влажности воздуха, шестнадцатичасовой день, освещение 5000 люксов, трехкратный (в течение недели) полив песчаной культуры питательной средей Хоглянда, содержащей микро- и макроэлементы, в остальные дни недели песок поливался дистиллированной водой. Содержание воды определялось по отношению к свежей массе (весовой процент) после высушивания нарезанных желудей в течение 48 часов при 105°C. В стратифицированных желудях содержание воды изучалось в вышеуказанной среде, содержащей 35 - 40% воды в свежей массе. Оценка состояния здоровья желудей проводилась при их нарезании.

Наличие ингибитора роста в кислой эфирной фракции водных экстрактов из желудей определялось при помощи теста удлинения колеоптиля пшеницы (сорт Ostka Chłopicka), после бумажной хроматографии (Wh 3) экстрактов, предварительно очищенных сольвентом: изопропанол-аммиак-вода (10 : 1 : 1).

Полученные результаты позволяют сделать следующие выводы:

1) Холодная стратификация желудей *Q. borealis* Michx. при температурах в границах 1° - 10°C наиболее успешно проходит при 5°C. Начало и конец прорастания приходятся при этой температуре на 92 и 144 день стратификации. При температурах в границах 1° - 5°C эпикотили не удлиняются.

2) Холодно-теплая стратификация, во время которой желуди стратифицировались сначала при температуре 3°C в течение 70 дней, а потом при температуре 20°C, делает возможным очень энергичную всхожесть и рост эпикотилей всех здоровых желудей, наступающие почти сразу после появления корней. Начало и конец прорастания приходится в этих условиях на 70 и 90 день стратификации (70 дней 3°C + 20 дней 20°C).

3) Из всех примененных в этом опыте температур холодного периода стратификации, предшествующего стратификации либо высеву при температуре 20°C, наиболее эффективной оказалась температура 5°C. Она обеспечивает самую высокую жизнеспособность семян, и все здоровые желуди прорастают.

4) Желуди способны к прорастанию в сырой среде при температуре 20°C без предварительной холодной стратификации. Прорастание в этих условиях проходит менее энергично и медленнее, чем при температурах в границах 1° - 5°C, однако начинается на 60 - 80 дней раньше, и растут тогда не только корни, но и эпикотили. Желуди, лишенные околоплодника, либо околоплодника и наружной ворсинчатой семенной оболочки, прорастают при температуре 20°C в тот же срок, что целые желуди. Сеянцы, полученные из неохлажденных желудей, проявляют признаки физиологической карликовости роста по сравнению с сеянцами из семян, стратифицированных перед повышением температуры в течение 49 дней при пониженной температуре.

5) Удаление околоплодника и наружной ворсинчатой семенной оболочки способствует повышению процента семян, прорастающих во время стратификации как при пониженной (3°C), так и при повышенной температуре (20°C). При температуре 3°C удаление околоплодника либо околоплодника вместе с наружной семенной оболочкой ускоряет прорастание соответственно на 20 либо 30 дней.

6) Смачивание желудей перед стратификацией в течение 7 дней в ежедневно сменяемой дистиллированной воде при температуре 3°C является неэффективным приемом, ухудшающим жизненность и всхожесть семян.

7) Независимо от применяемого способа стратификации (холодная или холодно-теплая) содержание воды в стратифицированных желудях при ее высоком начальном содержании (48% свежей массы) повышается во время стратификации и прорастания только незначительно (на 7 - 8% в прорастающих семенах).

8) У неподсушенных после опадания с дерева и нестратифицированных еще желудей обнаружена в ворсинчатой мягкой наружной семенной оболочке и в лишенных ее семенах высокая концентрация неидентифицированного до настоящего времени ингибитора роста, возможно абсцисиновой кислоты.

Fot. K. Jakusz

Cunninghamia lanceolata