ARBORETUM KÓRNICKIE
ROCZNIK XXX — 1985

BOLESŁAW SUSZKA

Conditions for after-ripening and germination of seeds and for seedling emergence of English yew (*Taxus baccata* L.)*

Abstract

Suszka B. 1985. Conditions for after-ripening and germination of seeds and for seedling emergence of English yew (*Taxus baccata* L.) Arboretum Kórnickie 30: 285 - 338.

When the initial warm phase of stratification of yew seeds runs at a cyclically alternating temperature followed by chilling — germination and seedling emergence is abundant in the next warm period. It was found for seeds originating from Poland that alternations of 15°~20°C are more effective in that phase than those of 15°~25°C found most effective by Devillez in Belgium. The duration of individual cycles was extended to 1+1 days/cycle instead of 12+12 hours/cycle, but even at 8+8 days/cycle the seeds germinated though in a somewhat lower percentage. The initial warm phase should last 6 - 6½ months and the subsequent chilling should be extended until the seeds start to germinate i.e. to 4 - 4½ months. After that the pretreated seeds can be placed for up to 12 weeks at −1°C to freeze the medium and to stop germination, but only 2 - 4 weeks are advisable. The seeds germinate and form seedlings in a high percent within the first 2 - 4 weeks at 20°C and with some delay at 3°~20°C (16+8 hours/cycle), whether they were treated by frost or not.

Key words: alternating temperature, freezing, stratification, thermal phases

Address: B. Suszka, Institute of Dendrology, 62-035 Kórnik, Poland.

INTRODUCTION

In appropriate ecological and climatic conditions English yew (*Taxus baccata* L.) regenerates in Poland, the seeds being dispersed from the female trees to greater distances mainly by some species of birds (Bartkowiak 1970, Bartkowiak and Zieliński 1973, Jackowski 1972, Król 1978). Seeds form only where female trees coexist with the male ones. On the other hand some authors report

* This work was partially supported by grant MR II-7, from the Polish Academy of Sciences.

such phenomena as lack of any regeneration or mass dying of young yew seedlings in places, where yew is native or in some reserves (Król 1978). There is a growing interest in saving this close to extinction tree species and some attempts have been undertaken by nurserymen, biologists and foresters to study the biology of its sexual regeneration.

Seeds of the English yew, as well as of other species of the genus *Taxus*, are characterized by a delayed germination, caused simultaneously by a bony though not impermeable (as was formerly thought) seedcoat, and by a minute and not fully developed and deep dormant embryo. The initial length of the embryo in ripe seeds is 1.2 - 1.8 mm (Le Page-Degivry 1973a, Devillez 1976), while the total length of the surrounding female gametophyte is 5 - 6 mm. Dormancy of the embryo seems to be removed by washing out or transforming into a bound, inactive form of a growth inhibitor (ABA — abscisic acid) and by the activation of gibberellins, the latter process being possible only at a low temperature acting for 2 - 3 months (Le Page-Degivry 1973a, 1973b, Le Page-Degivry and Garello 1973). Isolated embryos do not differentiate in *in vitro* embryo culture but their presence is necessary for the callusing of the female gametophyte tissue (Zenkteller and Guzowska 1970).

In natural conditions and within the geographic range of the species seeds dispersed in late summer and in autumn do not germinate earlier than in the second spring after dispersal of ripe seeds (Wappes 1932, Anonim 1948, Heit 1969), and a part of the seeds germinates in the next spring or even later. Yew seeds sown in the nursery immediately after removal of the fleshy aril or in the spring after one winter of dry storage do not germinate before the next spring, not infrequently even 1 or 2 years later (Tyszkiewicz 1949, Krüssmann 1964, Lamb et al. 1975). The opinion expressed sometimes that fresh seeds sown immediately after collection and cleaning germinate mostly in the first spring (Tyszkiewicz 1949, Janson 1975) was not confirmed in stratification and sowing experiments (Tyszkiewicz and Dąbrowska 1953).

Barton (1939) studying the behaviour of yew seeds in controlled conditions has found for *T. cuspidata* seeds that at first a warm stratification is necessary at any temperature within the 20 - 25°C range (optimum at 20°C) acting 3 months, followed by a cold stratification at 1 - 5°C (optimum at 5°C). Heit (1969) was the first to find that stored seeds of some *Taxus* species including the English yew should be sown in nursery conditions in the summer not later than end of June. Such treatment assures in the soil (when mulched and shadowed) the necessary sequence of one warm and one cold periods before the next spring, when they start to germinate very early, as soon as the soil is defrosted. The seedlings emerge above ground level in early spring.

The opinion that yew seeds need for germination at first a coldmoist period (treatment) followed by a warm and after that again by a cold one (Lamb et al. 1975, Suszka 1978) was not confirmed experimentally. Devillez (1976) has shown that the first cold period is absolutely ineffective in overcoming the dormant condition and only the next warm thermal phase followed by a cold one makes germination of seeds possible. It was even found, that cold only stratification of yew seeds does not permit germination even after 4 years of such treatment, though the seeds remain viable and healthy (Rudolf 1974), like the dry seeds (Heit 1967), which retain viability even after 5 - 6 years of sealed storage at 1 - 2°C.

Heit (1969) was also the first to recognize that imbibed *T. baccata* seeds need a long period of warm temperature not shorter than 5 - 7 months, but only 2 - 4 months of a cold one. In this way at least 7 - 11 months of stratification should pass until the seeds are ready for germination in controlled conditions or in the mulched ground.

It was Devillez (1976, 1978) who found that during the warm phase of stratification of *T. baccata* seeds temperature should alternate daily (12+12 hours) from 15° to 25°C for 6 months and that the cold phase should last 3 months (at 5°C). The latter period should not be extended, because the seeds start to germinate between the end of the 3rd and 4th months. The slow course of germination can however be shortened to 3 weeks, when after the cold phase the seeds are subjected to a daily alternating (12+12 hours) temperature 10°~20°C, more effective than any other constant temperature in the range 10°~30°C or the alternating temperatures 15°~25°C or 20°~30°C. Devillez (1978) has also found that daily alternating temperatures alone (5°~15°C, 5°~20°C, 5°~25°C, 5°~30°C, 5°~35°C and 5°~40°C) acting even 12 months are similarily ineffective as 5°C (12 months) or 15°~25°C (6 months) in removing dormancy of *T. baccata* seeds. Growth of embryos in one-phase conditions is seriously arrested or nonexistent.

The aim of this work, started in 1966, in which the recommendation of Devillez (1976) were respected from the time of their publication, was to study the behaviour of *T. baccata* seeds of Polish origin i.e. from the eastern limit of the range of this species. It was decided to observe the growth of embryos, and not only germination of seeds but also seedling emergence at first when treated at constant temperatures but later at more differentiated cyclical temperature alternations than those applied by Devillez, especially in the first warm phase of seed treatment. Another aim of this study was to find whether the 12+12 hour thermal cycles could be replaced by those of longer duration. Finally it was planned to test, if the cold phase of seed stratification could be interrupted by freezing of the medium, to transfer germination of the pretreated seeds to a later date when necessary.

MATERIAL AND METHODS

GENERAL REMARKS

The studies on conditions favouring after-ripening, germination and seedling emergence of the English yew (*Taxus baccata* L.) seed were performed in the years 1966 - 1984. They are composed of experiments numbered 1 - 9. In all these experiments the same methods have been applied, though their application varied, depending on the design of the individual experimental series.

Experiments 1 - 5 were devoted to finding sequences of constant stratification temperatures of varying duration, permitting after-ripening and germination of yew seeds. As a criterion of dormancy cessation changes of the embryo : female gametophyte index (in short: embryo index) and germination of seeds were used.

In experiments 6 - 9 only warm-followed-by-cold sequencies have been applied with alternating daily temperatures in the initial phase. The whole treatment consisted of 3 phases: warm and alternating for the initiation of growth of the embryos, (phase I), cold for continuation of growth of the embryo and after-ripening (phase II), and various constant or alternating temperatures for germination and/or seedling emergence (phase III). It was Devillez (1976) from Belgium who found experimentally the high efficacy of alternating temperatures in phase I of seed treatment for the germination of the English yew seeds in phase III.

In experiments 7 - 8 also emergence of seedlings from seeds sown in a standard medium was studied, when the boxes with the sown seeds were held from the start in controlled temperature/time conditions or when the seeds were sown after the 2 first phases of treatment in stratification conditions. These observations were accompanied by studying changes of the embryo index and of the course of germination. In experiment 9 the possibility of storing after-ripened seeds by freezing was investigated.

SEED MATERIAL

Seed origin: Seeds for all experiments were collected exclusively in the Kórnik Arboretum. In spite of being at the eastern limit of the natural range of the species, the English yew grows well there and regenerates abundantly thanks to advantageous ecological conditions and the presence of birds responsible for its dissimination: the blackbird (*Turdus merula*) and the nuthatch (*Sitta europaea*) (Bartkowiak 1970, Bartkowiak and Zieliński 1973). Seeds were collected with arils from single trees to obtain more uniform seed populations,

sometimes mixed seed lots originating from several known trees were used. Detailed data on the seed material used are given in the descriptions of individual experiments.

METHODS

Collection, cleaning, predrying and storage of seeds: With the exception of experiment 5 (succesively repeated collection from September until November) only ripe, olive green to nearly black seeds in red arils were collected. Without permitting any fermentation they were mixed with sand and rubbed in cloth sacks until the seeds were completely freed from the aril tissue. The thin outer seed coat was completely removed by this treatment. After washing out any traces of the pulp the seeds were dried in a shaded place at room temperature for some (mostly 10) days until their moisture content decreased to 7 - 10% (fresh weight basis). After that they were stored in sealed bottles at $-1°$ or $-3°$C, until they were used for the experiments.

Estimation of moisture content of seeds: The seeds were weighed before and after drying at 105°C lasting 24 hours and the moisture content was expressed as % of fresh weight.

Estimation of seed viability: The cutting test (4 × 50 seeds) was used and seeds with a white or greenish embryo and white and firm "endosperm" (female gametophyte) were regarded as viable. It was however difficult to distinguish the embryo from the "endosperm" in freshly collected and still not dried seeds, in experiment 9 most viable seeds contained a glassy "endosperm" because of an extremely dry and warm period preceding collection. Also seeds non-germinated in germination or seedling emergence tests were subjected to cutting, for recognition of the reasons of their behaviour. Seeds treated with H_2SO_4 were later tested by the cutting method.

Estimation of the embryo index: The initially minute though already differentiated embryos elongate and all their dimensions increase only in appropriate thermal- and moisture conditions under air access, finally just before germination their length is equal to the axial length of the female gametophyte filling tightly the hard seed coat. The imbibed seeds were cut longitudinally, the embryo was isolated and measured under a binocular microscope and in the same way the length of the female gametophyte was estimated. When greenish embryos occured, they were measured and the data were calculated separately

for seeds with white and greenish embryos. For each estimation 4×10 embryos were used and the data were put into the formula:

$$\text{Embryo index} = \frac{\text{length of the embryo} \times 100}{\text{length of the female gametophyte}} \%$$

The final result is calculated as mean % value from all 4 replicates.

Treatment of seeds with sulphuric acid: In experiments 4 and 5 the seeds were soaked in concentrated H_2SO_4 to scarify the seedcoats. The treatment lasted 1, 2 or 3 hours, after that the seeds were washed thoroughly in running tap water for 10 min.

Stratification of seeds: The clean dry seeds were mixed with a moist standard medium of peat mull and sand, 1 : 1 by volume. This seed + medium mixture was placed in 250 ml glass jars (each for 50 seeds) closed by aluminium sheets with 3 ventillation holes. At regular time intervals i.e. every week at 20°C or at the alternating temperatures or biweekly at 3°C the seeds were taken out of the medium on a sieve. The germinating seeds with the radicle at least half as long as the seed were counted and removed, as well as the decaying seeds. When necessary the lacking moisture was supplemented and the whole was thoroughly mixed. Each stratification variant was repeated 4 times (4×50 seeds), in experiment 8 (seed lot 450 only) exceptionally 3 times (3×25 seeds).

Seedling emergence tests: The seeds were sown in plastic boxes (20×20×8 cm) in holes pressed to the depth of 1 cm in the standard sand : peat medium. In experiment 8 the sowing medium was enriched with macro- and microelements by mixing with Azofoska, a compound mineral fertilizer (33 g/100 l of the medium). Each box contained 2×50 seeds, each experimental variant was replicated 4 times (4×50 seeds). The boxes were covered with transparent lids provided with ventillation holes and were placed at 20°C or at alternating temperature 3°~20°C (16+8 hours/day). In the test chamber a single 40 W incandescent bulb was sufficient for a totally normal green discoloration of the hypocotyls, the cotyledons and the first needles of the emerging seedlings. After emergence of the first seedlings the lids were removed and the surface of the medium was watered not at weekly intervals but always when necessary.

During seedling emergence first the bowed hypocotyl became visible above the sowing medium. After that the seed was lifted from the medium by the growing hypocotyl and finally the hypocotyl took an erect position and the seed coat was shed by the enlarging cotyledons. In any of these phases of seedling growth decaying of the plantlets could be observed, and their actual number decreased. Therefore only the total number of seedlings was taken for the presentation of the course of seedling emergence including all actually visible types of growing

plantlets and the decaying ones. Only in such a way the total seedling emergence could be demonstrated.

Temperature changes and alternations during stratification: In experiments 1-5 constant temperatures have been applied for longer periods and various combinations of warm (e.g. 20°C) and cold (e.g. 3°C) periods of different duration and in varying sequence were tested. The aim of such experimental design was to imitate and to simplify the long-lasting warm summer periods and cold autumn-and-winter periods acting in natural conditions on seeds burried in the soil.

In experiments 6-9, with reference to the findings of Devillez (1976), various cyclic alternations of temperature have been applied during the first phase of stratification of seeds or even of seedling emergence tests, with the aim to make the growth of the embryos within the seeds possible. In contrast to the 24-hours (12+12 hours) cycles of Devillez 48-hour cycles (24+24 hours) were applied. In this way only one instead of two temperature changes had to be performed daily, always at the same hour. In one experiment (exp. 8) longer cycles have been tested: 4-days (2+2 days), 8 days (4+4 days) and 16 days (8+8 days). For practical reasons these temperature changes were made possible by transferring jars with the stratified seeds or boxes with the sown seeds from one chamber with the higher temperature to another chamber with the lower temperature of the cycle, and this was repeated as many times as was provided by the design of the experiment.

Temperatures during the germination test and seedling emergence tests: In experiments 6-9 after stratification at cyclically alternating temperature (for embryo growth) followed by a cold phase (for breaking of dormancy) a final stratification test or seedling emergence test was performed either at 3°C or 20°C (constant) or at 3°~20°C (16+8 hours/day, cyclically alternating), to enable an energetic germination of seeds and/or a fast growth of the seedlings. Here also temperature changes were obtained by transferring jars with the stratified seeds or boxes with the sown seeds from one chamber to another.

Freezing of the medium with the stratified seeds: In order to stop the final stages of the after-ripening process the jars containing the medium : seed mixture were placed at −1° or at −3°C for periods lasting from 2 to 12 weeks (experiment 9), when the germination of the first seeds became visible. After termination of each freezing period the medium was defrosted by placing the jars for 24 hours at 3°C, followed by an increase of temperature to 20°C to perform the germination tests in a stratified condition at this temperature.

DETAILED DESCRIPTION OF THE EXPERIMENTAL DESIGNS

A. ALL PHASES OF SEED TREATMENT AT CONSTANT TEMPERATURES

Experiment 1 (Lab. file No. 103)

Aim of the experiment: To study the efectiveness of long lasting, cold only stratification as well as warm-followed-by-cold stratification either followed or not by a period of moderately high temperature.

Seed material: Seeds were collected on 19 - 21 September 1966, when the arils were red and the outer surface of seeds dark green. The seeds were immediately cleaned and were used for the experiment as fresh seeds on 24th October 1966.

Design of the experiment (fig. 1):

— cold stratification: at 1°, 3°, 5° and 10°C for 52 weeks (4 variants),

— warm-followed-by-cold stratification: at 25°C for 5, 8, 11 and 14 weeks, after that for the next 46 weeks at 1°, 3°, 5° and 10°C (16 variants),

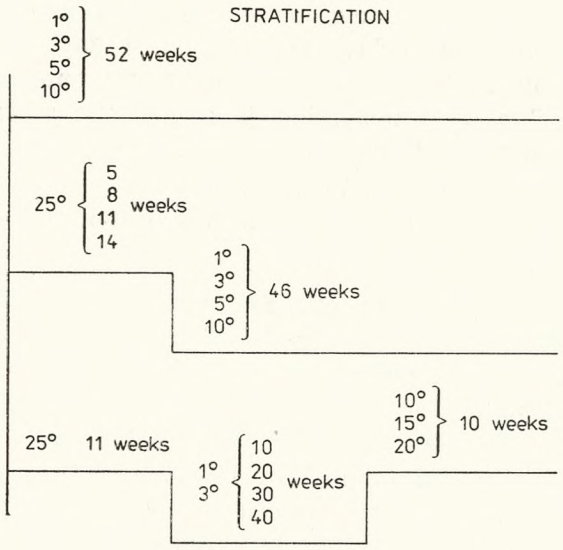

Fig. 1. *Taxus baccata* L. Design of experiment 1 started in 1966 (seed lot 103)

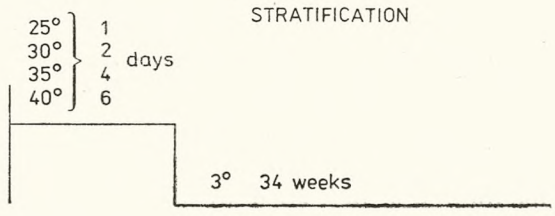

Fig. 2. *Taxus baccata* L. Design of experiment 2 started in 1967 (seed lot 127)

— warm stratification at 25°C lasting 11 weeks, after that cold stratification at 1°C or 3°C for 10, 20, 30 or 40 weeks, followed by a germination test in stratification conditions at 10°, 15° or 20°C (24 variants).

Experiment 2 (Lab. file No. 127)

Aim of the experiment: To find whether germination of seeds could be obtained at low temperature preceded by a short period of warm treatment at various temperature levels up to 40°C.

Seed material: Collected from 1 tree on 13th November 1967. The seeds were cleaned on November 14th, the experiment was started immediately.

Design of the experiment (fig. 2): The seeds were stratified for 1, 2, 4 or 6 days at 25°, 30°, 35° or 40°C, after that they were transferred to 3°C for the next 34 weeks (16 variants). During the warm phase moisture of the medium was inspected daily.

Experiment 3 (Lab. file No. 218)

Aim of the experiment: To study the efectiveness of a warm-followed-by-cold stratification of fresh and predried seeds at various temperatures, the cold phase was followed after gradually extended periods by a germination test at 20°C.

Seed material: Seeds were collected from 5 trees from October 23rd to November 8th 1972. Cleaning was performed after collection,

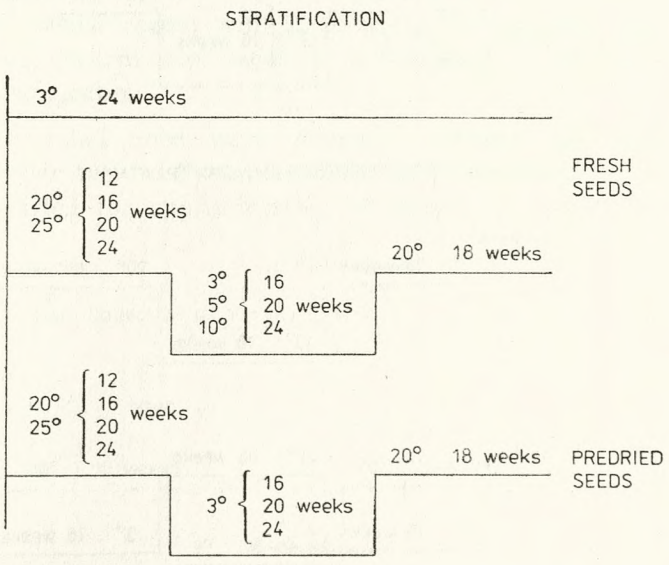

Fig. 3. *Taxus baccata* L. Design of experiment 3 started in 1972 (seed lot 218)

latest on 11th November 1972. The seeds were used for the experiment immediately after cleaning when still fresh (moisture content 33,1%) but also on 12th December 1972, after partial drying at 15 - 18°C to 6,4% of moisture content. Moisture content of seeds was estimated during both the warm and the cold stratification. Viability of seeds was tested by the cutting test before the experiment and after termination of each phase of treatment.

Experimental design (fig. 3):
Fresh seeds:
— cold only stratification at 3°C for 54 weeks (control, 1 variant),
— warm stratification at 20° or 25°C for 12, 16, 20 or 24 weeks, followed by cold stratification at 3°, 5° or 10°C lasting 16, 20 or 24 weeks, after that the temperature of stratification was raised to 20°C for the next 18 weeks to trigger germination (72 variants).
Predried seeds:
— as above, but cold phase of stratification only at 3°C (24 variants).

Experiment 4 (Lab. file No. 254)

Aim of the experiment: To study the effect of stratification in the following sequence of long-lasting temperature phases: cold-warm--cold-warm on the elongation of embryos and germination of seeds,

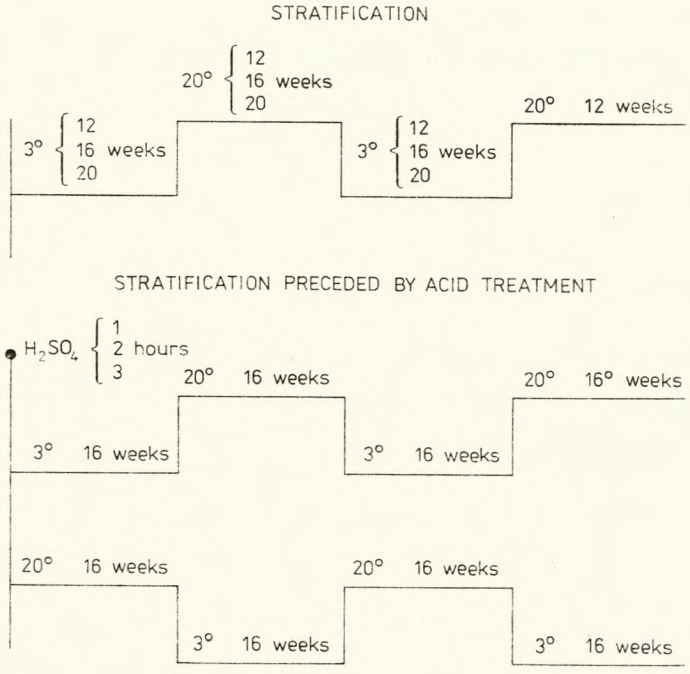

Fig. 4. *Taxus baccata* L. Design of experiment 4 started in 1974 (seed lot 254)

whether combined or not with scarification of the seed coat with conc. H_2SO_4.

Seed material: A mixture of seeds collected from 3 trees from 26th September till 5th October 1974. After cleaning the seeds were dried to 9,4% of moisture content and were stored in sealed bottles at −1°C from 17th October till 15th November 1974. Seeds used in the series with the acid treatment were stored for further 11 days at 5°C.

Experimental design (fig. 4):

Stratification experiments:

— stratification at 3°C for 12, 16 or 20 weeks, followed by 20°C for 12, 16 or 20 weeks, followed by stratification at 3°C lasting 12, 16 or 20 weeks, followed always by 12 weeks at 20°C (27 variants),

Changes of moisture content were studied as well as seed viability (cutting test) before and after each phase of treatment.

Stratification preceded by acid treatment:

— seeds were stratified following 2 basic stratification designs, each phase lasting 16 weeks: 3°/20°/3°/20°C
20°/3°/20°/3°C

Before starting first phase of stratification the seeds were treated for 1, 2 or 3 hours with concentrated sulphuric acid. After rinsing in running tap water lasting 10 minutes the seeds were placed in stratification conditions.

Experiment 5 (Lab. file No. 308)

Aim of the experiment: To study the effect of the date of seed collection on seeds treated with sulphuric acid and after that stratified in a warm-cold-warm-cold sequence of temperatures in the subsequent phases of stratification.

Seed material: Seeds were collected at 10-day intervals in the time from September 14th to November 25th 1977 (8 collections). The cleaned seeds were dried for approximately 10 days to 6.7 - 9.6% of moisture

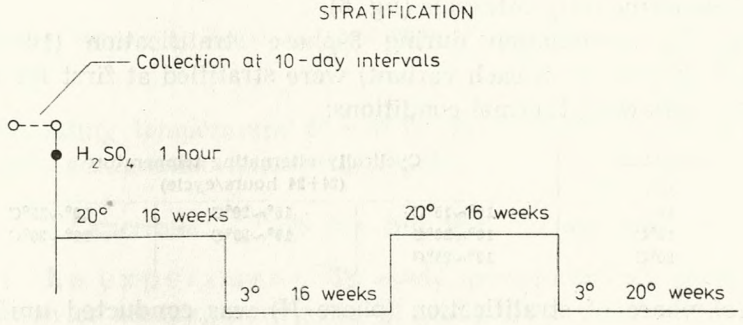

Fig. 5. *Taxus baccata* L. Design of experiment 5 started in 1977 (seed lot 308)

content. After drying the seeds were immersed for 1 hour in concentrated H_2SO_4, afterwards they were rinsed in running tap water and stratified at 20°/3°/20°/3° (16+16+16+20 weeks), the total duration of stratification being 68 weeks (fig. 5).

B. FIRST PHASE OF SEED TREATMENT AT ALTERNATING TEMPERATURE

Experiment 6 (Lab. file No. 362)

Aim of the experiment. To study growth of embryos within seeds at various cyclically alternating temperatures and germination of seeds during stratification consisting of 3 thermal phases: first with temperature constant or cyclically alternating, followed by a constantly cool phase and terminated by a period of cyclically alternating temperature.

Seed material: Seeds were collected from many trees from 22nd to 28th September 1977. After cleaning and drying to 7.8% of moisture content the seeds were stored in sealed bottles for 2 weeks at 3°C and afterwards for 3 months at −3°C, until the experiments were started. At that time moisture content of seeds was 8.1%, viability 100.0% (cutting test), embryo index 43.7%.

Design of the experiment (fig. 6):

a. Study of embryo growth (15 variants):
The seeds were stratified for 6 months in the following thermal conditions:

Constant temperature	Cyclically alternating temperature (24+24 hours/cycle)			
5°C	5°~10°C	10°~15°C	15°~20°C	20°~25°C
10°C	5°~15°C	10°~20°C	15°~25°C	
15°C	5°~20°C	10°~25°C		
20°C	5°~25°C			

and at 15°~20°C for 6 months followed by 4 months at 3°C. During stratification the embryo index was estimated for each thermal variant at monthly intervals (fig. 6).

b. Study of germination during 3-phase stratification (10 variants). The seeds (4×50 in each variant) were stratified at first for 6 months in the following thermal conditions:

Constant temperature	Cyclically alternating temperature (24+24 hours/cycle)		
10°C	10°~15°C	15°~20°C	20°~25°C
15°C	10°~20°C	15°~25°C	20°~30°C
20°C	10°~25°C		

The cool phase of stratification (phase II) was conducted uniformly at 3°C for 4, 5 or 6 months and was followed by the last phase at a cycli-

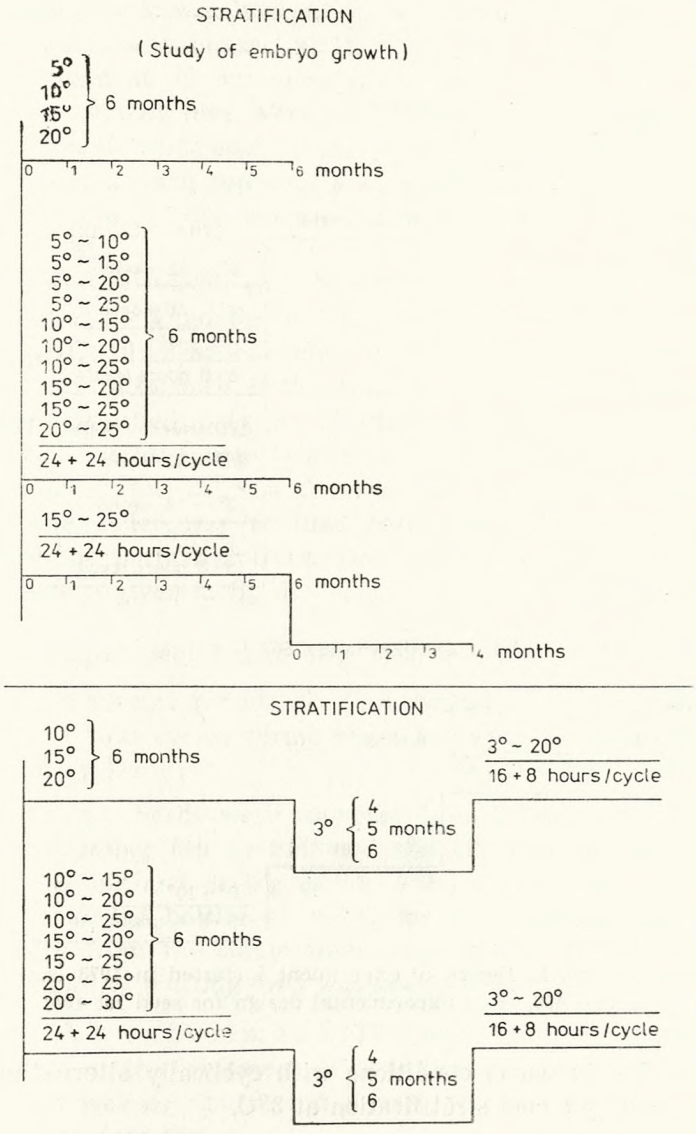

Fig. 6. *Taxus baccata* L. Design of experiment 6 started in 1977 (seed lot 362)

cally alternating temperature 3°~20°C (16+8 hours/cycle), to trigger an energetic germination (phase III) (fig. 6).

Experiment 7 (Lab. file Nos. 406, 407 and 408)

Aim of the experiment: To study germination of seeds during stratification or seedling emergence of seeds sown in laboratory conditions after a 2-phase stratification. In both series stratification was per-

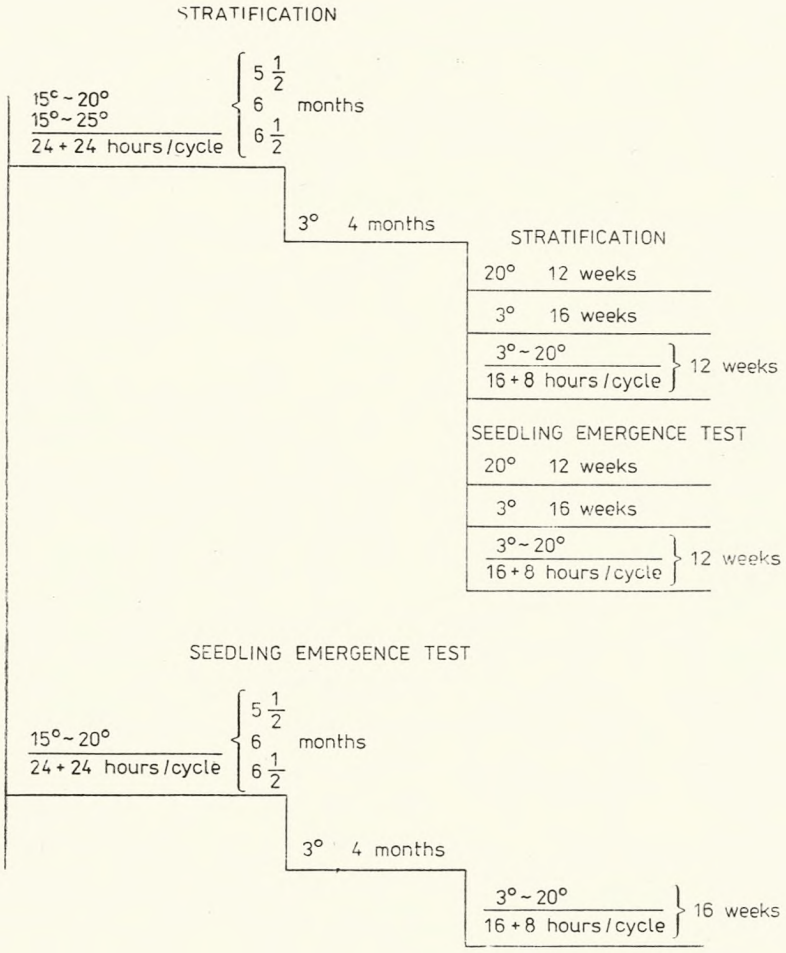

Fig. 7. *Taxus baccata* L. Design of experiment 7 started in 1978 (seed lots 406, 407 and 408, basic experimental design for seed lot 408)

formed at first in warm conditions with cyclically alternating temperature, followed by a cool stratification at 3°C.

Seed material: The seeds were collected from 3 trees in the time from 9th to 13th October 1978 and were used simultaneously but separately in 3 experiments basing on the same design, differing only in the number of variants in phase III of the treatments. The cleaned and dried seeds were stored for 2 months in sealed bottles at −3°C at 7.9%, 7.8% and 9.8% of moisture content respectively. Their viability was 100.0%.

Design of the experiment (fig. 7): The seeds of each tree were stratified at two cyclically alternating temperature regimes: 15°~20°C and 15°~25°C (24+24 hours/cycle) for 5.5, 6 and 6.5 months (phase I)

after that temperature was lowered to 3°C for 4 months (phase II). In exp. 408 one series with phase I at 15°~20°C was added, but the seeds were not stratified at all but were sown in boxes and until the very end of the experiment they were left untouched, notwithstanding the subsequent thermal treatments during phases I, II and III. In all experimental variants to each replicate a constant number of 20 seeds was added for estimation of the embryo index after phase I and II of the thermal treatment.

In exp. 408 in the 15°~20°C and 15°~25°C series lasting 6 months (followed in phase II by 4 months at 3°C), the seeds were stratified at 3°, 20° and 3°~20°C (16+8 hours/cycle) for the next 12 or 16 weeks (phase III) but they were also sown at the same thermal systems. Seeds of the lot 407 were stratified only at 3°~20°C, and those of lot 406 in the 15°~20°C series (phase I) were stratified and sown at 3°~20°C (phase III), and in the 15°~25°C series they were only stratified at 3°~20°C. These differences resulted from varying amounts of seeds collected from the particular trees. The basic design (reduced for seed lots 407 and 406) is given in fig. 7.

Experiment 8 (Lab. files Nos. 449, 450, 451)

Aim of the experiment: To find whether it is possible to replace the 24+24 hour cycles during phase I of seed treatment by longer lasting thermal cycles.

Seed material: Seeds were collected from 3 trees (2 trees in the time between October 6th to 9th, and the 3rd tree on 23rd October 1979). After cleaning and drying to 7.8 - 9.8% of moisture content they were stored in sealed bottles at −3°C for 2 - 3 months. The embryo indices of seeds from the single trees were 44.6%, 37.2% and 36.7% respectively, viability (cutting test) was always 100.0%.

Design of the experiment: The seeds were held during the first two phases of the treatment in stratification conditions. In phase III stratification was continued, but a part of the seeds was used for the seedling emergence test.

In phase I the cyclically alternating temperature 15°~20°C was applied for 6.5 months, but 4 types of cycles were simultaneously used:

24+ 24 hours/cycle	(1+1 day = 2 days)
48+ 48 hours/cycle	(2+2 days= 4 days)
96+ 96 hours/cycle	(4+4 days= 8 days)
192+192 hours/cycle	(8+8 days=16 days)

Phase II was a cold stratification at 3°C, which in all series lasted 4 months uniformly.

In phase III seeds from each series were stratified at 3°, 20°C and

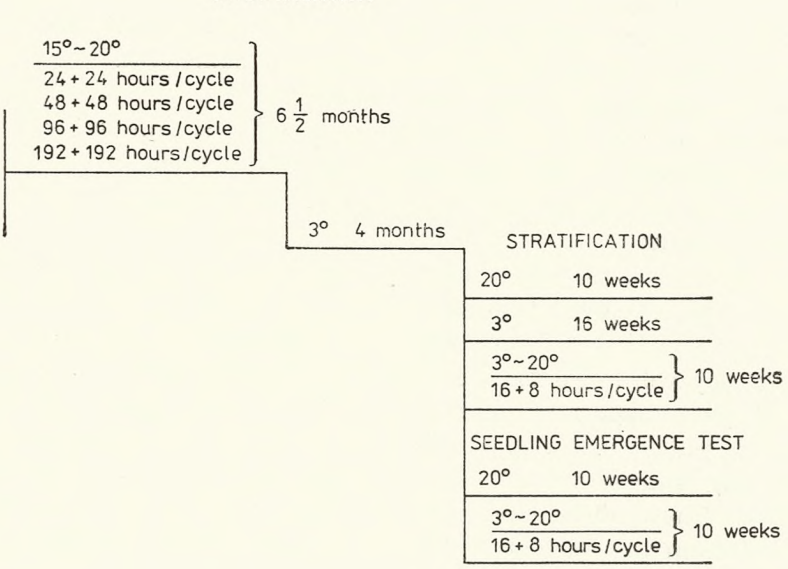

Fig. 8. *Taxus baccata* L. Design of experiment 8 started in 1979 (seed lot 451)

3°~20°C (16+8 hours/cycle). They were also sown in an fertilized sand/peat medium and the sowing boxes were held at 20° and at 3°~20°C (16+8 hours/cycle) (fig. 8).

After termination of stratification phases I and II embryo indices were estimated. Seeds for these measurements were at the start of the experiment added to each replicate of each experimental variant (2×10 seeds).

Experiment 9 (Lab. file No. 559)

Aim of the experiment: To study the possibility of suspending the after-ripening process in stratified seeds by interrupting the cold phase (phase II) of stratification by a period at a temperature below 0°C.

Seed material: The seeds were collected from 3 trees on 27th September 1982. The cleaned seeds were mixed and dried to be stored later for 4 months at −1°C in sealed bottles, until the experminent was started. At that time their moisture content was 10.3%, viability was 100.0%.

Design of the experiment: The seeds were at first stratified for 6 months at a cyclically alternating temperature 15°~20°C (24+24 hours/cycle) after that the cool stratification at 3°C was continued for 4 months i.e. until the seeds (2.6%) started to germinate. At that time the jars with the stratified seeds were transferred to refrigeration chambers with −1° and −3°C, where they remained for 2, 4, 6, 8, 10 and 12 weeks, to be brought back after that for the last 6 weeks to 20°C. At

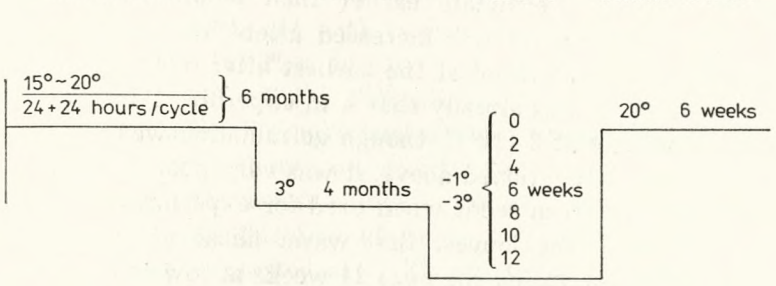

Fig. 9. *Taxus baccata* L. Design of experiment 9 started in 1982 (seed lot 559)

this latter temperature germination was continued and terminated (fig. 9). The control variant was not frozen at all. Embryo indexes were estimated before and after phase I (alternating) and phase II (cool) of seed stratification.

RESULTS

A. STRATIFICATION SYSTEMS BASED ON CONSTANT TEMPERATURES

In experiment 1 germination of seeds never surpassed 1.3% whether stratification was long-lasting (52 weeks) and constantly cool at 1°, 3°, 5° or 10°C, or warm-followed-by-cold 25°/1°C, 25°/3°C, 25°/5°C or 25°/10°C notwithstanding the duration of the warm phase up to 14 weeks and of the cold one up to 46 weeks. Seeds did not germinate even when 11 weeks of stratification at 25°C were followed by up to 40 weeks at 1° or 3°C and after that by a period at 10°, 15° or 20°C. All these treatments were ineffective.

When in experiment 2 a 34-week chilling was preceded by a short (maximally 6 days) warm stratification at temperatures in the range 25 - 40°C, none of so treated seeds germinated.

In experiment 3 the first warm phase of stratification at 20° or 25°C was extended up to 24 weeks. The following cool phase at 3°, 5° or 10°C was stopped latest after 24 weeks and after that the temperature of stratification was again increased to 20°C for the next 18 weeks. In this last warm phase germination was obtained in some experimental variants, as well as in some warm-followed-by-cold only variants with an extended cold phase. However, with the exception of 2 variants (8.5% and 8.5%) the germination percentage never exceeded 3.0%. The 2 variants mentioned above were: 25°/10°C and 25°/10°/20°C. The conclusion which could be drawn from the results of experiment 3 as well as of the former experiments is that yew seeds do not germinate at all at 20° or 25°C acting up to 24 weeks because of their deep dormant seeds and the underdeveloped embryos. During the following cool phase

they do not start to germinate earlier than before 16 - 19 weeks of chilling. When temperature was increased after the termination of this cool phase germination started at the earliest after one week of the elevated temperature. It was already shown in experiment 1 that yew seeds are able to germinate at 3 - 10°C, though germination was scattered over a long period and as mentioned above, it was very poor.

Embryo index of fresh seeds when used for experiment 3 was 33.1%. After termination of the longest first warm phase of 24 weeks it did not exceed 40.6% and during the next 24 weeks at low temperature (phase II) in non-germinating, i.e. in the majority of seeds it remained at the 42.0 - 43.1% level. It became clear that the applied thermal conditions did not promote the growth of embryos embedded inside of the female gametophyte (improperly called "endosperm"). Viability of seeds was always high and it remained at the 78.0 - 97.5% level, depending on the time/temperature experimental variant. Moisture content of fresh seeds was 33.1%, after 24 weeks of the first warm phase it grew to 40.6% at 20°C and to 43.3% at 25°C. After the next 24 weeks at 3°C it was still 43.1 - 43.2%.

These data indicate that the hard seed coat does not hinder water access to the living tissues of the seeds. However, when fresh and relatively moist seeds were used for stratification, changes of moisture content were slow at both the applied temperatures. High initial viability did not change in many variants of experiment 3 nor in the former experiments, even when temperature changed 3 times and the total duration of all 3 stratification phases was 66 weeks, and when the longest extention of both the initial warm and the following cold phase was 24 weeks.

In experiment 4 four thermal stratification phases followed each other in the sequence: warm-cold-warm-cold or cold-warm-cold-warm at 3° and 20°C. These phases lasted 12 - 20 weeks each and only in the fourth phase the longest variant was 16 weeks. In none of the 36 variants of this experiment seeds germinated more than 1.0%. The hard seed coat was never an obstacle for the penetration of water into the initially dry (9.4% of moisture content) seeds. At 20°C it increased in the first 5 days to 29.7%, after 12 weeks at this temperature it was already 35.4%. The highest level of moisture content was found after termination of the experiment i.e. after 64 weeks and it was 40.5%. Provention of seeds from germination was not caused by a decreased viability, since the latter never fell in any of the stratification variants below 98.5%. The embryo index of the non-germinating seeds was at the most 49.0%. All this means that dormancy of seeds treated in the way given above even over 64 weeks could not be broken by any of the temperature sequences applied during stratification.

In the experimental series where stratification (20°/3°/20°/3° or

3°/20°/3°/20°C lasting 16+16+16+16)=64 weeks was preceded for 1, 2 or 3 hours by a treatment with concentrated sulphuric acid viability remained high (98.8 - 100.0%), and moisture content increased from the initial 8.9% up to 34.6 - 40.5% depending on the variant (31.9 - 36.8% during the first phase). Moisture content was always higher in variants starting with a warm phase and acid-treated longer than for 1 hour. Embryo index did not surpass 41.5% in variants starting at 3°C and it did not depend on the duration of the acid treatment. When the first phase of stratification started at 20°C two types of embryos could be observed at the end of this experiment: Most embryos (50.0 - 90.0%) were greenish and their indices were 83.0%, 68.0% or 66.7% respectively, this decrease depending on the extension of the acid treatment from 1 to 3 hours. The remaining embryos were white and minute, and their indices did not go beyond 45.3%. Despite this differentiation germination of seeds in the acid-treated series was never better than 1.2%. When comparing this result with the 1.0% of seeds germinating in the many experimental variants with seeds not acid-treated — the only profit resulting from this treatment was a better water access to the living seed tissues and some promotion of the embryo growth and development.

In both series of experiment 4 germination was always obtained in the second warm phase. This has caused us to assume that dormancy of embryos was surely broken during the cool phase of stratification, separating the warm phases while growth of embryos which germinated has occurred during the first warm phase. When the first phase of stratification was a cold one, dormancy could not be broken, because in such conditions growth of embryos was impossible. The problem was to find conditions (temperature, duration etc.) during the 3 subsequent phases (warm-cold-warm) which could trigger a mass germination in phase III of seed treatment.

In experiment 5 an attempt was made to elucidate the effect of the date of seed collection on their ability to germinate in standard conditions. The latter comprised always the warm-cold-warm-cold sequence of 16-week phases (20° and 3°C) with only the last phase lasting 20 weeks instead of 16. Seeds were collected at 10-day intervals from mid-September till the end of November. Before starting stratification the fresh seeds were dried and treated with concentrated sulphuric acid for 1 hour.

Generally speaking the later stratification started, the higher was the seed viability after termination of the last stratification phase. Germination was very low, maximally 4.5% and seeds germinated mostly in the first 2 - 3 weeks of the second warm phase, as in experiment 4. Embryo indices of the non-germinating seeds were low (38.5 - 47.0%), depending on the date of collection, but without any regular trend in this context. Only in the last and latest variant 21.5% of seeds were

found with radicles 0.5 mm long, still far from being regarded as germinated.

From this experiment the conclusion was drawn, that seeds did not germinate because of an improper thermal system. The acid treatment was harmless, at least for seeds collected latest.

B. STRATIFICATION SYSTEMS WITH CYCLICALLY ALTERNATING TEMPERATURE IN THE FIRST PHASE OF TREATMENT, FOLLOWED BY A COLD PHASE AND TERMINATED BY THE GERMINATION PHASE AT A CONSTANT OR CYCLICALLY ALTERNATING TEMPERATURE

In 1978 we became acquainted with results of Devillez (1976), who has applied successfully alternating temperature during the first (warm) phase of stratification of the English yew seeds. Best variants were 15°~25°C and 10°~20°C in 12+12 hours cycles, extended up to 6 months. When transferred to 5° or 10°C but especially to 5°C, the seeds started to germinate in between the 3rd and 4th month of chilling. During the first warm-alternating stratification phase the embryo indices did not surpass 45.0%. During the cold phase embryo growth was continued until the seeds started to germinate after termination of the after-ripening process in each seed separately. It seems that growth of embryos and after-ripening run simultaneously during chilling, but it is clearly visible, that this growth became possible only by the alternating and not constant temperature of the initial warm phase. In this way 9 - 10 months were necessary to start germination of the stratified yew seeds.

Since 1978 starting with experiment 6, alternating temperature in the warm phase of stratification has been applied also in our experiments. Our experimental designs differed from those applied by Devillez in the following way:

— seeds were collected always directly from the trees,
— stratification was performed in a moist sand : peat mull medium,
— 48-hour cycles (24+24 hours/cycle) have been applied instead of the 24-hour ones (12+12 hours/cycle) as in studies of Devillez,
— a bigger variety of cycle-types was tested,
— in some variants seeds were not only stratified but also sown, in laboratory conditions, either from the start of the thermal treatment or starting with the last (third) phase following the warm and cool stratification phases.

Optimal temperature in the warm phase I of seed pretreatment (experiment 6)

In the experiment 6 alternating temperature was applied for the first time in our study on embryo elongation and germination of *T. baccata* seeds. The results are presented in table 1. Even after 6 months

Table 1

Number (in %) of white and greenish embryos in seeds of *Taxus baccata* L. before and after 6 months of stratification at constant and cyclically alternating temperatures (24+24 hours/cycle). The embryo index expresses the length of the embryo as % of the length of the female gametophyte (Experiment 6)

Temperature of stratification	White embryos		Greenish embryos	
	%	index %	%	index %
	100.0 before	43.7 stratification	–	–
5°	100.0	44.1	–	–
5°~10°	100.0	45.1	–	–
5°~15°	100.0	43.8	–	–
5°~20°	100.0	43.8	–	–
5°~25°	100.0	41.0	–	–
10°	100.0	44.6	–	–
10°~15°	100.0	44.7	–	–
10°~20°	57.5	42.7	42.5	60.1
10°~25°	84.7	45.8	15.4	55.2
15°	97.4	48.8	2.6	88.6
15°~20°	15.4	46.0	84.6	70.2
15°~25°	10.0	48.5	90.0	61.7
20°	100.0	45.4	–	–
20°~25°	100.0	52.4	–	–

Best results are underlined

of stratification the embryos did not elongate within the seeds at any constant temperature in the range between 5° and 25°C. They remained white and their index was below or around 50%. From the applied 10 experimental variants with alternating temperature only in 4 variants did the embryos become greenish and started to elongate in the 3rd and 4th month of the action of alternating temperature. All other embryos in these variants remained white and viable, and only in the 20°~25°C variants some elongation of the still white embryos was observed. The embryo indices of the greenish embryos in the optimal variants, especially at 15°~20°C and 15°~25° increased after 6 months in these conditions up to 70.2% and 61.7% respectively and 84.6% and 90.0% of seeds contained such activated embryos. The effectiveness of the two other embryo-activating temperature variants 10°~20°C and 10°~25°C was much lower, especially that of the latter one.

In another series, experiment 6, the behaviour of the embryos was followed in bi-monthly intervals during the second (cool) phase of seed stratification. For the first (warm) phase alternating cycles of 15°~25°C were chosen, the cool phase was performed at 3°C. The results are shown in Table 2. The high percentage of greenish and elongated embryos achieved during the alternating warm stratification phase did not change during the first 3 months of the cold phase of stratification. However, in the time between the 3rd and 5th month of chilling the so far rather stable (within some limits) proportion of white and activated

Table 2

Number (in %) of white and greenish embryos in sound seeds and number of decayed seeds of *Taxus baccata* L. after the warm phase of stratification at 15° ~ 25°C (24+24 hours/cycle) lasting 6⅓ months, and after the subsequent cold phase of stratification at 3°C lasting 1, 3 or 5 months (Experiment 6)

Phase I of seed stratification at 15° ~ 25°C					Phase II of seed stratification at 3°C					
Duration months	White embryos		Greenish embryos		Duration months	White embryos		Greenish embryos		Decayed seeds %
	%	index %	%	index %		%	index %	%	index %	
6⅓	7.5	49.7	80.0	64.2	0					12.5
					1	17.9	47.2	64.2	68.7	17.9
					3	15.0	53.4	72.5	74.4	12.5
					5	67.5	65.7	20.0	92.1	12.5

greenish and elongated embryos changed and at the end of this period the percentage of the white ones increased from less than 20% to 67.5% and even they started to elongate (index 65.7). The greenish embryos now with a very high index (92.1%) became much less frequent and their percentage decreased to 20.0%. In fact it should be taken into account that after 4 months of the cold phase seeds started to germinate at the applied low temperature and for embryo measurements only the non-germinating seeds were used after separation of the already germinating ones.

It seems therefore that growth of embryos occurs mainly during the warm phase of stratification and that at that time alternating temperature is absolutely necessary for their elongation. During subsequent chilling seeds after-ripen at first and growth of the embryos is again resumed shortly before germination, the latter being only the last stage of embryo growth.

Germination of seeds during stratification in experiment 6 is shown in table 3 and fig. 10. Here phase I (warm) of stratification is fixed uniformly for 6 months, running either at constant temperatures in the range 10 - 25°C or at cyclically alternating ones (48-hour cycles). The duration of the cool phase II (3°C) was differentiated (4, 5 and 6 months), it was always followed by phase III at alternating temperature 3°~20°C (16+8 hours/cycle). In this way start of the germination period had to be found during phase II and it was hoped to estimate germinative capacity during phase III.

It became evident (table 3) that in all variants with phase I at constant temperature germination was nil (10°C), below 5.0% (15°C) or in the best case below 10.0% (20°C). Also very low was the effectiveness of one variant with alternating temperature: 10°~15°C. In all other variants of the latter type germinative capacity was differentiated. Seeds germinated most intensively when temperature alternations during phase

Table 3

Germinative capacity (in %) of *Taxus baccata* L. seeds during 3-phase stratification with phase I at constant or cyclically alternating temperature (24+24 hours/cycle) lasting 6 months, phase II at 3°C lasting 4, 5 or 6 months and phase III at 3°~20°C (16+8 hours/cycle) lasting 10 weeks. Additional data (in %) concern seeds germinating before phase III and decaying seeds. (Experiment 6)

Duration of stratification phases:	Phase I:	6 months			Mean
	Phase II:	4 months	5 months	6 months	
	Phase III:	10 weeks	10 weeks	10 weeks	
		%	%	%	%
Temperature (°C) during phase I of stratification:	10°	0.0 (0.0) [0.5]	0.0 (0.0) [5.5]	0.0 (0.0) [6.5]	0.0
	10°~15°	1.0 (0.0) [2.0]	0.5 (0.5) [7.0]	0.0 (0.0) [3.5]	0.5
	10°~20°	34.5 (0.0) [0.0]	33.5 (18.0) [11.5]	23.0 (17.5) [9.5]	30.3
	10°~25°	20.5 (0.0) [31.0]	20.0 (5.0) [1.5]	13.0 (7.0) [16.5]	17.8
	15°	3.5 (0.0) [14.0]	2.5 (2.5) [12.5]	1.5 (1.5) [6.0]	2.5
	15°~20°	80.5 (0.0) [12.0]	74.5 (44.0) [13.0]	75.0 (67.5) [14.0]	76.7
	15°~25°	79.5 (0.0) [12.5]	66.5 (22.5) [24.5]	83.0 (56.5) [7.0]	76.3
	20°	7.0 (0.0) [19.0]	4.5 (0.0) [8.0]	1.5 (1.5) [12.0]	4.3
	20°~25°	58.5 (0.0) [30.0]	41.5 (2.0) [17.0]	40.0 (16.0) [22.0]	46.7
	20°~30°	47.5 (0.0) [48.0]	36.5 (7.5) [38.5]	34.5 (15.5) [27.5]	39.5
Mean:		33.2	28.0	27.2	

Additional information: ( ) % of seeds germinating till the end of phase II
[ ] % of seeds decaying till the end of phase III
underlined: optimal variants

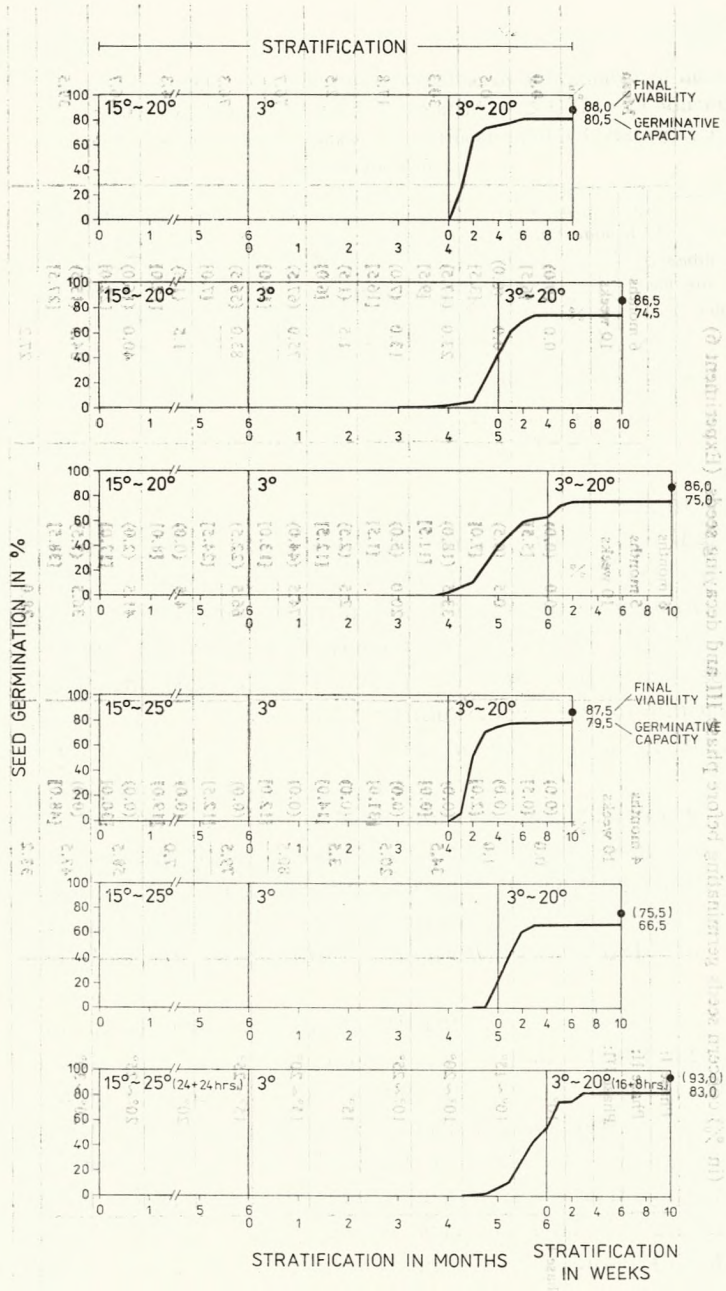

Fig. 10. Course of germination of *Taxus baccata* L. seeds (seed lot 362) subjected to a 3-phase stratification. In phase I 48-hour cycles 15°~20°C and 15°~25°C (24+24 hours/cycle) were applied, in phase III 24-hour cycles 3°~20°C (16+8 hours/cycle). Duration of phase II was differentiated (Experiment 6)

I were based on 15°C as the lower temperature, and the obtained levels did not differ whether the amplitude of temperatures in each cycle was 5° or 10°C. Both best variants: 15°~20° and 15°~25°C were equally effective, assuring 79.5 - 80.5% of germinating seeds.

From the additional data in table 3 (in square brackets) we can conclude, that when phase III was started after 5 or 6 months of cold phase II, the number of seeds germinating before this change of temperature was already 22.5 - 44.0% after 5 months in the mentioned above best variants and 56.5 - 67.5% after 6 months, the other seeds germinating in phase III at the alternating temperature. Only during the shortest chilling phase II (4 months) seeds did not germinate at all but their readiness to germinate was revealed by the alternating temperature 3°~20°C in daily cycles. In this way only after 6+4 months of stratification all capable seeds germinated in phase III.

The course of germination in the experimental series with phase I at 15°~20° and 15°~25°C is shown in fig. 10. It is evident that yew seeds are able to germinate at 3°C (variants with phase II lasting 5 and 6 months) and if this phase were still more extended — probably all after-ripened seeds would germinate at this low temperature, though over a much longer time, presumably in about 10 weeks since the start of germination. When phase III was started after 4 months of phase II, i.e. after a period of chilling permitting complete after-ripening of seeds with the shortest demand for the action of low temperature — germination started promptly and thanks to the high participation (66%) of low temperature in each daily cycle after-ripening was continued and dormancy gradually terminated. Thanks to the high temperature of each cycle the already after-ripened seeds could germinate without delay.

The final conclusion from the data given above was that it should be possible to obtain seedlings from seeds prepared before sowing for germination in the way presented here i.e. after a 2-phase stratification lasting 6+4 months (15°~20°C or 15°~25°C followed by 3°C).

Thermal conditions of phases I and II of seed pretreatment and of germination or seedling emergence in phase III (experiment 7)

The application in experiment 6 of alternating instead of constant temperatures in the first phase of stratification of *Taxus baccata* seeds of Polish provenance has assured excellent results. Some questions however had still to be elucidated. The experiment 7 was therefore started in 1978 with the aim of finding the optimal duration of stratification phase I and to know the reaction of seeds when in phase III various temperatures would be applied. Another problem was the behaviour of seeds when after stratification comprising the first two phases they would be sown at controlled conditions (constant or alternating tempe-

Table 1

Percentage of white and greenish *Taxus baccata* L. seeds after phase I and phase I+II of stratification. Phase I was performed at cyclically alternating temperature 15°~20°C or 15°~25°C (24+24 hours/cycle), phase II always at 3°C. (Experiment 7)

Phase I of stratification		Phase II of stratification		White embryos	Greenish embryos	Decayed seeds
Temperature °C	Duration months	Temperature °C	Duration months	%	%	%
15°~20° (24+24 hours/cycle)	5½	–	–	67	3	
	6			42	53	5
	6½			25	75	0
	Mean:			32	65	3
	5½	3°	4	36	51	13
	6		4	14	86	0
	6½		4	27	65	8
	Mean:			26	67	7
15°~25° (24+24 hours/cycle)	5½	–	–	50	50	0
	6			35	52	13
	6½			20	71	9
	Mean:			35	58	7
	5½	3°	4	43	44	13
	6		4	26	74	0
	6½		4	21	65	14
	Mean:			30	61	9

The presented values are mean percents for 3 seed lots.

rature) or when they would be placed in the sowing medium before any thermal treatment. In the latter case all phases of the thermal treatment would be conducted on already sown seeds.

The results concerning only stratified seeds are shown in table 4 and figs. 11A, 12, 13 and 14. In table 4 and fig. 11A data are presented concerning the development of embryos after phase I (warm) and II (cold) of stratification. Because of the small basis for measurements (4×10 embryos for each seed lot and variant) the calculated mean values do not reflect clearly the general trends, when divided into groups with differentiated duration of phase I. However, the mean values of these numbers show these trends precisely: the number of white embryos decreases during phase II (the cool phase) slightly, while that of the greenish ones increases but to a lesser degree and some seeds decay. In other words — nearly all embryos changing their colour from white to greenish do so already during phase I (warm) and the white embryos which can be regarded as the not activated ones, nearly all remain white also during the subsequent 4-month chilling period. Embryos activated in the alternating temperature of phase I differ after that phase from the white embryos, being not only coloured but also longer. During the chilling phase at 3°C their growth accelerates and after 4 months their mean indices grow to the range 85 - 94%. This makes their germination possible. Indices of the white embryos remain

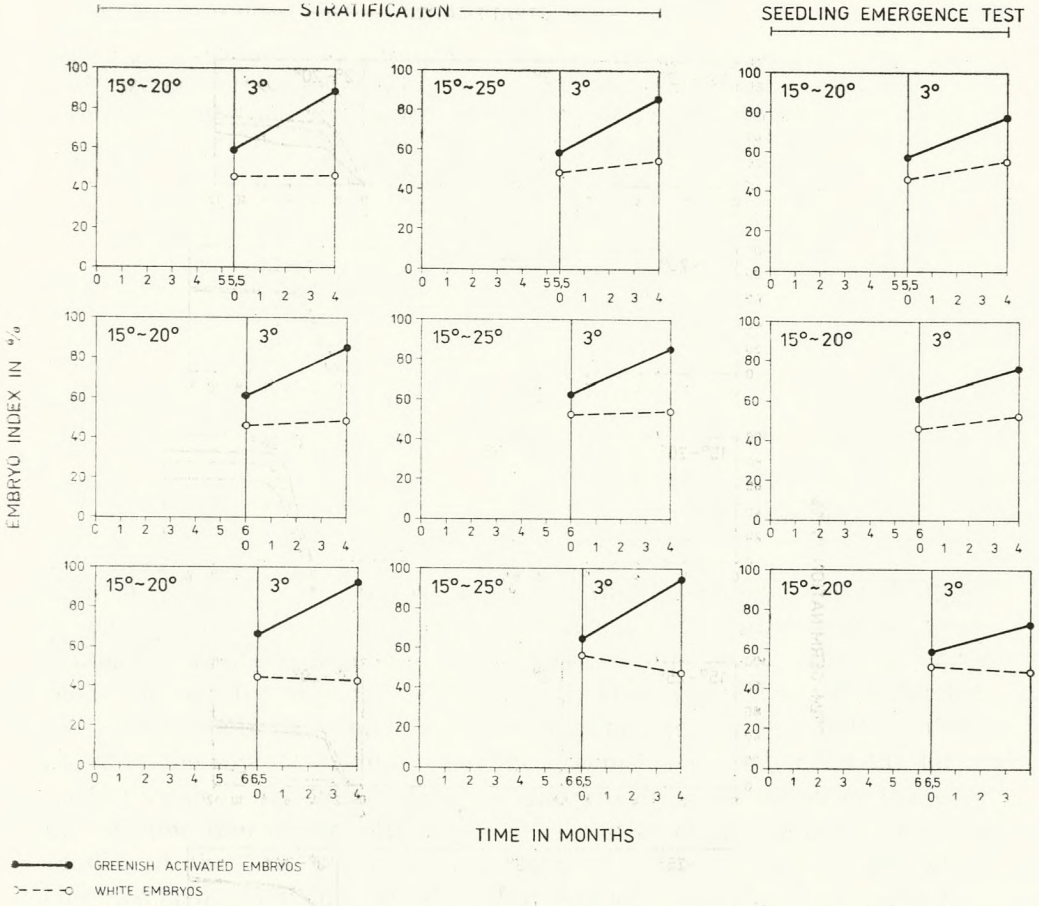

Fig. 11. Mean indices of *Taxus baccata* L. embryos before and after phase II of seed stratification (seed lots 406, 407 and 408). In phase I stratification was performed at 15°~20°C and 15°~25°C in 48-hour cycles (25+24 hours/cycle) for 5.5, 6 and 6.5 months (A). Similar data concern seed lot 408 sown from the very beginning in laboratory conditions (seedling emergence test) with 15°~20°C in phase I (B). (Experiment 7)

at the same time i.e. after 9.5 - 10.5 months of stratification (both phases) at a 42 - 54% level, only slightly bigger than of embryos at the start of the stratification phase I.

It is also interesting to know how do the embryos develop in seeds not stratified at all but sown from the start and subjected to the thermal treatments in the sowing medium. These data originate from one seed lot only (seed lot 408) and are presented in table 5 and in fig. 11B. For the same reason as above only mean values calculated for all durations of phase I can be regarded as indicators of the general trends. Even then it is not certain that the number of white embryos increases during chilling and that of the green ones decreases as can be seen in

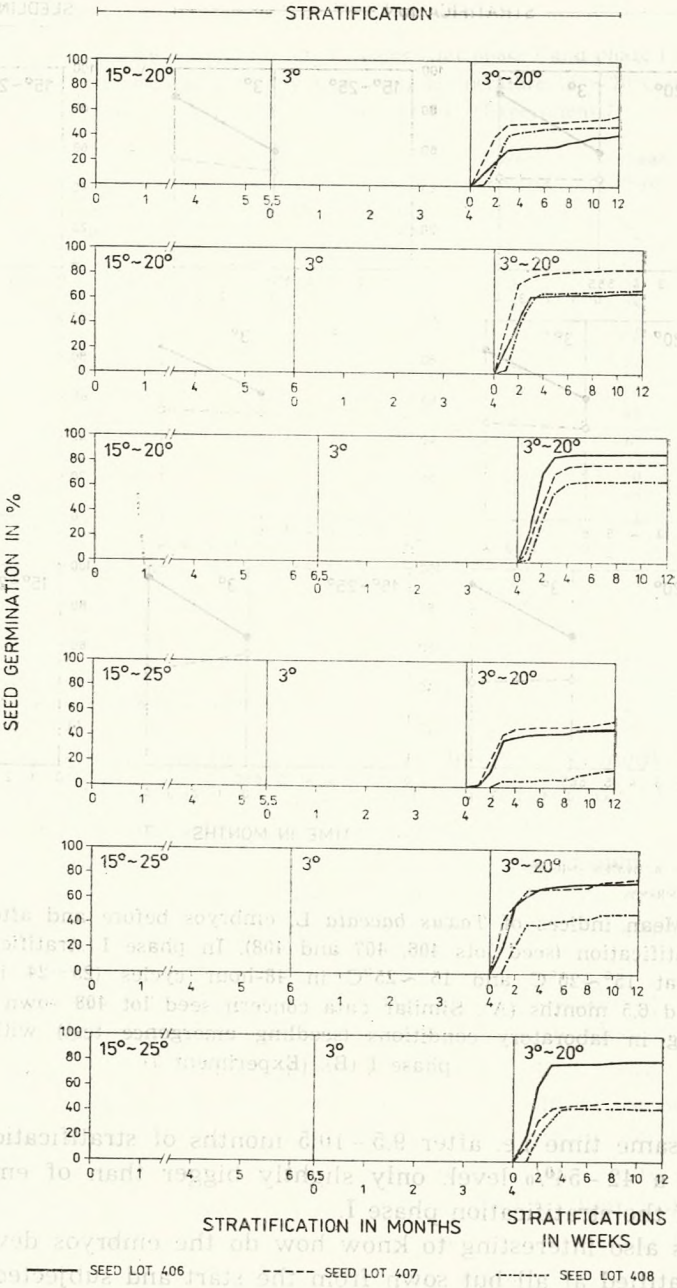

Fig. 12. Course of germination of *Taxus baccata* L. seeds (seed lots 406, 407 and 408) during a 3-phase stratification. In phase I 48-hour cycles 15°~20°C and 15°~25°C (24+24 hours/cycle) were applied, in phase III 24-hour cycles 3°~20°C (16+8 hours/cycle). Duration of phase I was differentiated. (Experiment 7)

Table 5

Percentage of white and greenish *Taxus baccata* L. embryos and of decayed seeds after phase I and I + II of thermal treatment of seeds not stratified but from the very beginning sown in laboratory conditions. Phase I was performed at cyclically alternating temperature 15° ~ 20°C, (24+24 hours/cycle), phase II at 3°C. Only one seed lot (408) was used for these measurements (Experiment 7)

Phase I of thermal treatment for the sown seeds		Phase II of thermal treatment for the sown seeds		White embryos	Greenish embryos	Decayed seeds
Temperature °C	Duration months	Temperature C°	Duration months	%	%	%
15°~20°C (24+24 hours/cycle)	5½	–	–	64	36	0
	6	–	–	24	76	0
	6½	–	–	39	32	29
	Mean:			42	48	10
	5½	3°	4	78	22	0
	6			44	56	0
	6½			37	42	21
	Mean:			53	40	7

table 5. What can be concluded is that the percentages of both types of embryos do not change seriously during the chilling phase when compared with the numbers at the start of this phase, and that more seeds can accidentally decay in phase II when phase I is too extended. The white embryos elongate much less during the 4-month chilling phase in the sowing medium than the greenish ones, but even the latter do not achieve the dimensions of embryos from seeds stratified only in the same thermal conditions. Embryo indices of the greenish embryos remain at the 71 - 76% level after phase II, while those of the white embryos achieve values of 47 - 54%. After phase I the corresponding numbers were 56 - 60% and 44 - 50% respectively. These data confirm the conclusion presented above that thermal treatments should be preferred which assure already in phase I the activation (greening, elongation) of as high a percentage of embryos as possible.

Basing on results of experiment 6 shown in table 1 the number of thermal variants of phase I has been reduced in experiment 7 to 2 only: 15°~20° and 15°~25°C. The latter variant was found already by Devillez (1976, 1978) as very effective, the former one with the smaller amplitude of temperature alternations proved so far equally effective in our studies.

Both temperature variants for stratification phase I were compared in experiment 7 in an experimental series for which 3 different seed lots (nos. 406, 407 and 408) were used, differentiated also was the duration of phase I (5.5, 6 and 6.5 months). After a uniformly long (4 months) chilling period (phase II) alternating temperature 3°~20°C (16+8 hours/cycle) was applied in phase III, with the aim to provoke germination still in the stratification conditions. The course of germination and the

Table 6

Germinative capacity (in %) of *Taxus baccata* L. seeds in phase III of 3-phase stratification with phase I at cyclically alternating temperature (24+24 hours/cycle) of 15°~20° or 15°~25°C lasting 5.5, 6 or 6.5 months, phase II at 3°C lasting 4 months and phase III at 3°~20° (16+8 hours/cycle) lasting 16 weeks (Experiment 7)

Duration of stratification phase I months	Phase I at 15°~20°C				Phase I at 15°~25°C				Overall mean
	seed lot 406 %	seed lot 407 %	seed lot 408 %	mean %	seed lot 406 %	seed lot 407 %	seed lot 408 %	mean %	%
5½	41.5	56.0	47.0	48.2	43.5	52.5	12.5	36.2	42.2
6	64.4	82.5	67.0	71.3	72.0	74.5	47.5	47.5	68.0
6½	86.5	78.0	64.5	76.3	79.0	47.5	42.5	56.3	66.3
Mean:	64.2	72.2	59.5		64.8	58.2	34.2		
Overall mean:		65.3				52.4			

Table 7

Germinative capacity (in %) of *Taxus baccata* L. seeds (seeds lot 408) in phase III of 3-phase stratification with phase I at cyclically alternating temperature (24+24 hours/cycle) lasting 6 months, phase II at 3°C lasting 4 months and phase III at 3°, 20° or 3°~20°C (16+8 hours/cycle) (Experiment 7)

Temperature in stratification phase I	Temperature in stratification phase III			Mean
	3°C	20°C	3°~20°C (16+8 hours/cycle)	
	%	%	%	%
15°C~20° (24+24 hours/cycle)	57.5	62.5	67.0	62.3
15°~25°C (24+24 hours/cycle)	30.0	36.0	47.5	37.8
Mean:	43.8	49.2	57.2	

obtained levels of germinative capacity and final seed viability are presented in fig. 12. Numerical data on germinative capacity are compared in table 6. It can be concluded that seed lots from individual trees can differ seriously in their reaction to the same stratification conditions, and that temperature alternations in 2-day cycles (24+24 hours/cycle of

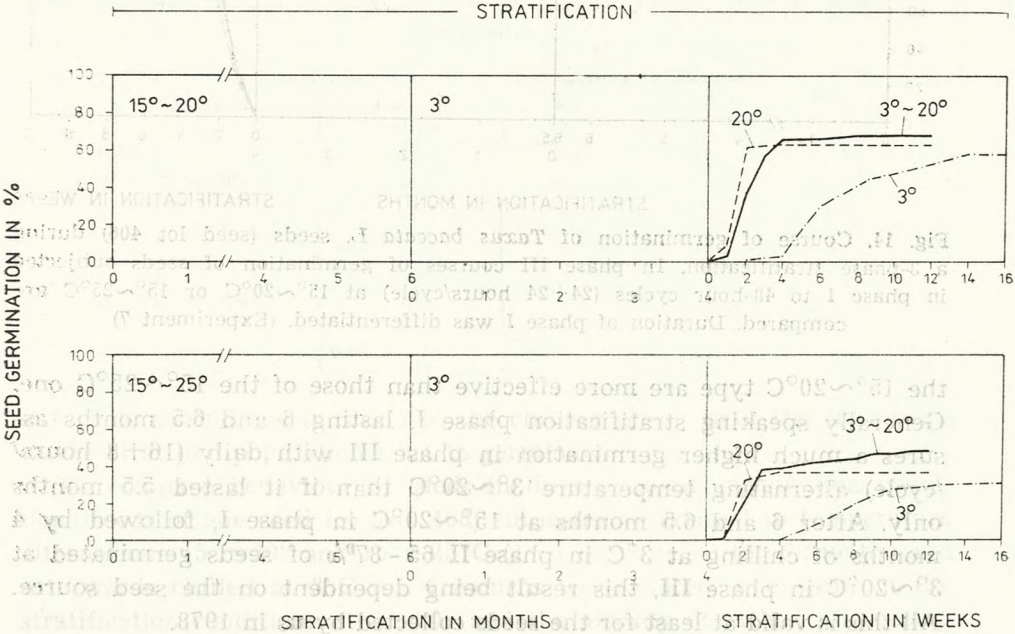

Fig. 13. Course of germination of *Taxus baccata* L. seeds (seed lot 408), during a 3-phase stratification. In phase I 48-hour cycles 15°~20°C and 15°~25°C (24+24 hours/cycle) were appplied. In phase III temperature was differentiated (3°, 20° or 3°~20°C, 16+8 hours/cycle). (Experiment 7)

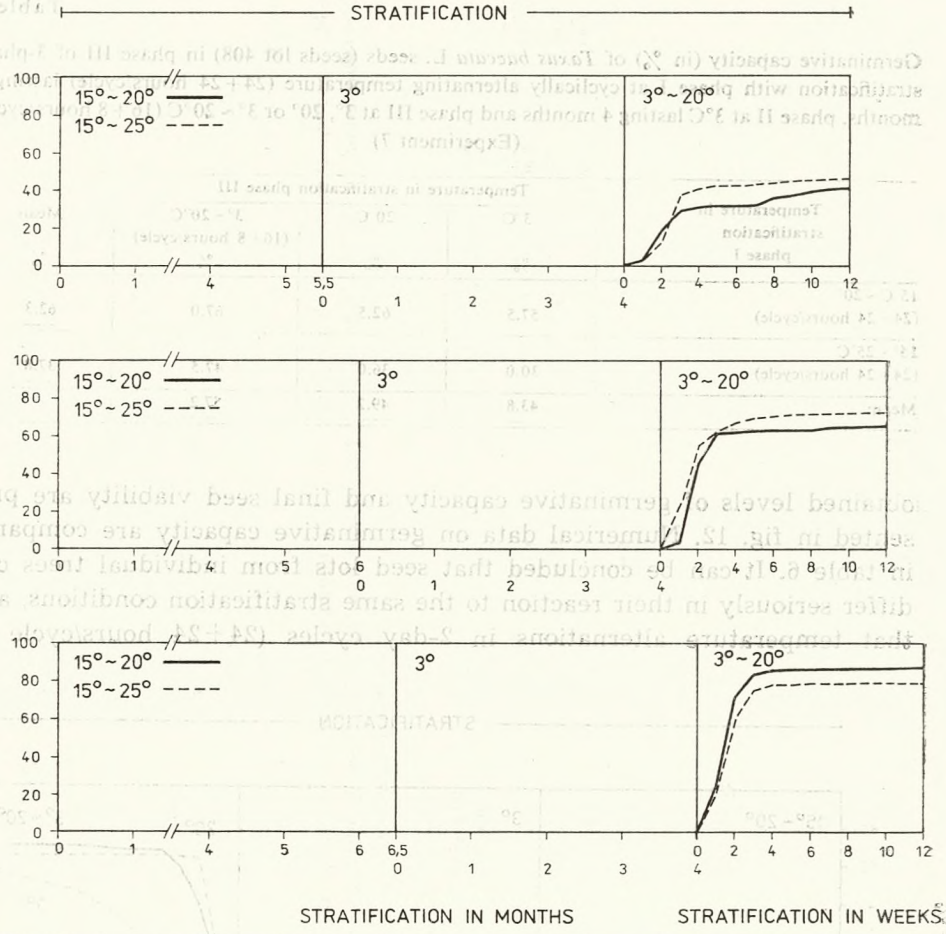

Fig. 14. Course of germination of *Taxus baccata* L. seeds (seed lot 406) during a 3-phase stratification. In phase III courses of germination of seeds subjected in phase I to 48-hour cycles (24+24 hours/cycle) at 15°~20°C or 15°~25°C are compared. Duration of phase I was differentiated. (Experiment 7)

the 15°~20°C type are more effective than those of the 15°~25°C one. Generally speaking stratification phase I, lasting 6 and 6.5 months assures a much higher germination in phase III with daily (16+8 hours/ /cycle) alternating temperature 3°~20°C than if it lasted 5.5 months only. After 6 and 6.5 months at 15°~20°C in phase I, followed by 4 months of chilling at 3°C in phase II 65 - 87% of seeds germinated at 3°~20°C in phase III, this result being dependent on the seed source. All this is valid at least for the seeds collected by us in 1978.

From the course of the germination curves it can be concluded that the 4-month chilling period was long enough or even a little too short, especially in the case of seed lot 408, where germination at 3°~20°C started always after a short 1-week lag. To avoid such situations it would

Table 8

Germinative capacity (in %) of *Taxus baccata* L. seeds in phase III of 3-phase stratification presented separately for various seed lots. Phase I was performed at cyclically alternating temperature (24+24 hours/cycle) of 15°~20°C or 15°~25°C lasting 5½, 6 or 6½ months, phase II at 3°C lasting 4 months and phase III at 3°~20°C (16+8 hours/cycle) lasting 16 weeks (Experiment 7)

Phase I of stratification		Seed lots		
Temperature °C	Duration months	406 %	407 %	408 %
15°~20°C (24+24 hours/cycle)	5½	41.5	56.0	47.0
	6	64.5	82.5	67.0
	6½	86.5	78.0	64.5
15°~25°C (24+24 hours/cycle)	5½	43.5	52.5	12.5
	6	72.0	74.5	47.5
	6½	79.0	47.5	42.5
Overall mean:		64.5	65.2	46.8

Table 9

Seedling emergence (in %) of *Taxus baccata* L. in phase III of seed treatment. Phase I of stratification was performed at cyclically alternating temperatures 15°~20°C or 15°~25°C (24+24 hours/cycle) for 5½, 6 or 6½ months, phase II at 3°C for 4 months. After that the seeds were sown at laboratory conditions and were held at 3°~20°C (16+8 hours/cycle) for the next 16 weeks (Experiment 7)

Stratification phase I		Seedling emergence of seed lots		Mean
Temperature °C	Duration months	406 %	408 %	%
15°~20°C (24+24 hours/cycle)	5½	22.0	33.0	27.5
	6	60.5	63.0	61.8
	6½	78.0	65.0	71.5
	mean:	53.5	53.7	
15°~25°C (24+24 hours/cycle)	5½		13.5	
	6		49.0	
	6½		55.0	
	mean:		39.2	

suffice to extend the duration of the chilling phase until the first seeds in the stratified population start to germinate.

To compare behaviour of seeds in differentiated germination conditions seeds of one seed lot (No. 408) subjected in phase I to temperature alternations 15°~20° and 15°~25°C (1+1 day cycles) for 6 months and afterwards chilled at 3°C for 4 months were placed when still in the stratification conditions at 3°, 20° and the already described alternating temperature 3°~20° (16+8 hours/cycle). The numerical data of this comparative experimental series are presented in table 7, the course of germination is shown in fig. 13. These data indicate a superiority of the 15°~20°C cycles over those of the 15°~25°C type, valid for all

Table 10

Seedling emergence (in %) of *Taxus baccata* L. in phase III of seed treatment. Phase I of stratification progressed at 15°~20°C or 15°~25°C (24+24 hours/cycle) for 6 months, phase II at 3°C for 4 months. After that the seeds were sown in laboratory conditions and were held at 20°C or at 3°~20°C (16+8 hours/cycle) for 16 weeks (seed lot 408, experiment 7)

Temperature of stratification phase I °C	Phase III after sowing in laboratory conditions		Mean
	20°C	3°~20°C (16+6 hours/cycle)	
	%	%	%
15°~20°C (24+24 hours/cycle)	63.0	63.0	63.0
15°~25°C (24+24 hours/cycle)	44.7	49.0	46.8
Mean:	53.8	56.0	

temperature variants of phase III. Most effective was the alternating temperature (3°~20°C), somewhat less effective is the constant temperature of 20°C. At 3°C germinative capacity was lowest, all this independently from the thermal cycles in phase I. From the germination curves in fig. 13 we can conclude, that at 3°C germination started late, after nearly 4 weeks, and progressed slowly. At 20°C nearly all seeds germinated in the second week, and after that germination was completely stopped. At 3°~20°C seeds started to germinate within the first week and till the end of the 4th week nearly all capable seeds germinated. In these thermal conditions resembling temperature fluctuations in the upper soil level in spring, seeds germinated best though germination was somewhat delayed, when compared with that at 20°C. At 3°~20°C the number of seeds decaying during phase III was also much less than that at 3° or 20°C.

On the example of seed lot 406 it is demonstrated (fig. 14) that an extension of the warm phase I of stratification to 6.5 months can be beneficial for the germination of yew seeds, when phase II at 3°C lasts until the seeds are ready to initiate it. So treated seeds germinated in stratification conditions in the first 4 weeks in about 80% or even more when temperature alternations (3°~20°C, 16+8 hours/cycle) were applied. In the optimal variant (6.5+4 months of pretreatment) seeds subjected to 15°~20°C in phase I germinated better than those treated at 15°~25°C. Basing on these results we can conlude that 15°~20°C acting for 6.5 months during stratification phase I can improve germination in phase III, at least in the case of yew seeds from some individual trees.

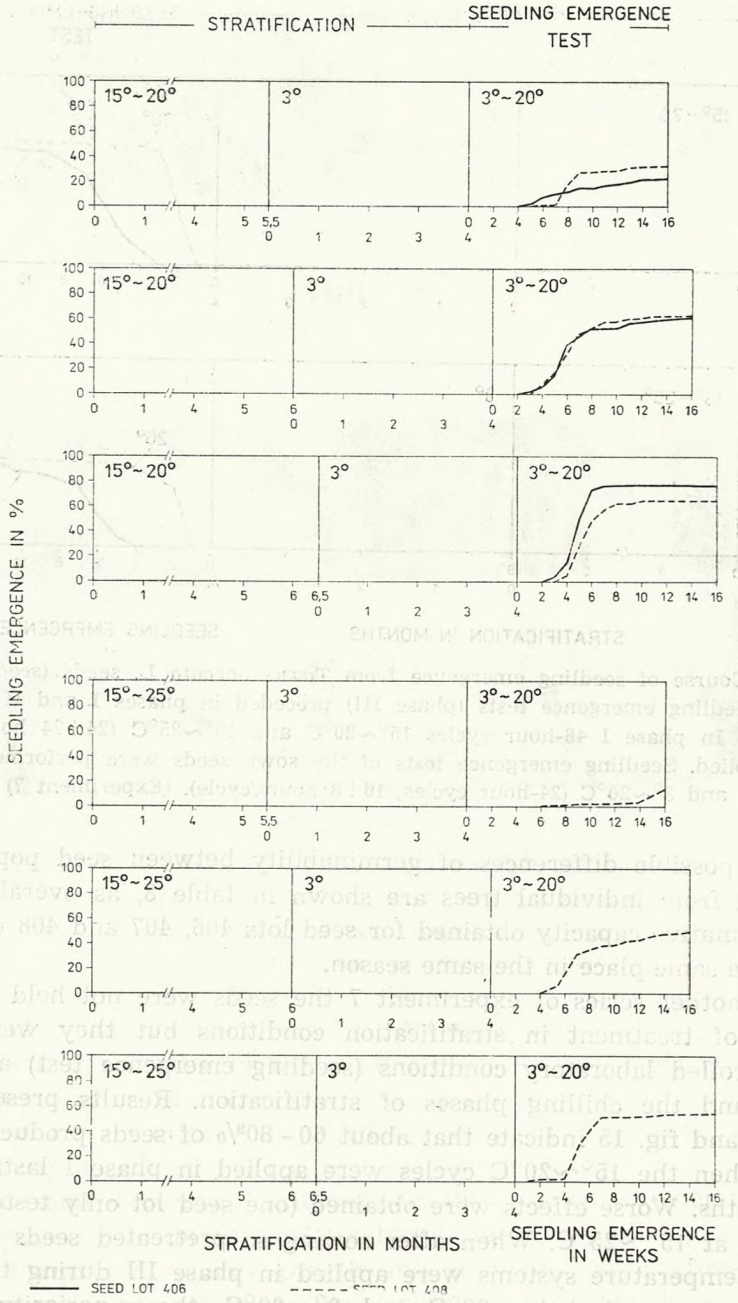

Fig. 15. Course of seedling emergence from *Taxus baccata* L. seeds during seedling emergence tests preceded in phases I and II by stratification. In phase I 48-hour cycles 15°~20°C and 15°~25°C (24+24 hours/cycle) were applied and the duration of this phase was differentiated. For the 15°~20°C cycles in phase I seed lots 406 and 408 are compared in phase III, for 15°~25°C only seed lot 408 was used. (Experiment 7)

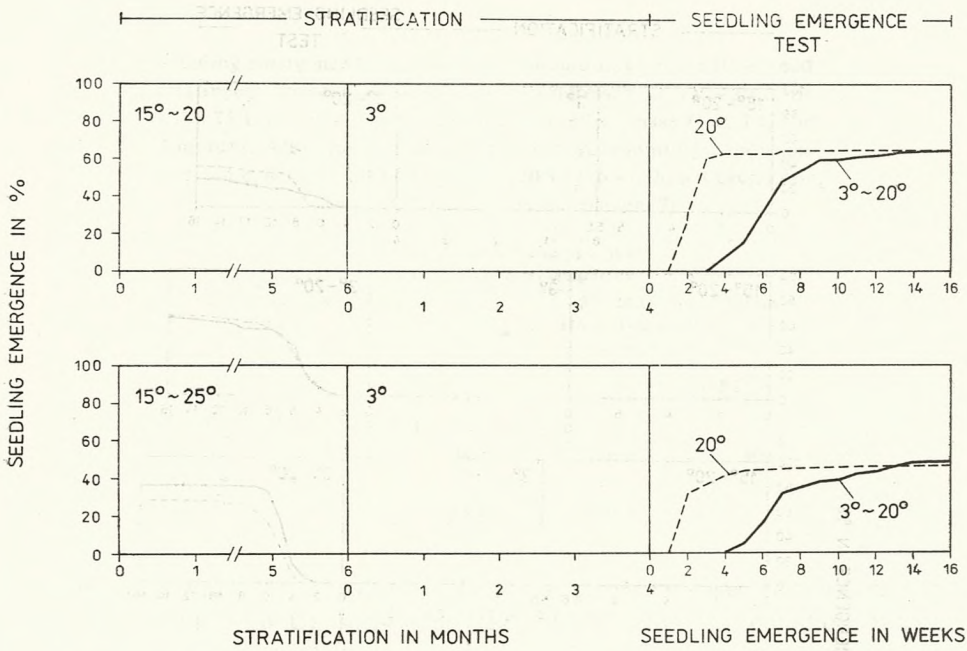

Fig. 16. Course of seedling emergence from *Taxus baccata* L. seeds (seed lot 408) during seedling emergence tests (phase III) preceded in phases I and II by stratification. In phase I 48-hour cycles 15°~20°C and 15°~25°C (24+24 hours/cycle) were applied. Seedling emergence tests of the sown seeds were performed at 20° and 3°~20°C (24-hour cycles, 16+8 hours/cycle). (Experiment 7)

The possible differences of germinability between seed populations deriving from individual trees are shown in table 8, as overall means of germinative capacity obtained for seed lots 406, 407 and 408 collected from the same place in the same season.

In another series of experiment 7 the seeds were not held in all 3 phases of treatment in stratification conditions but they were sown in controlled laboratory conditions (seedling emergence test) after the warm and the chilling phases of stratification. Results presented in table 9 and fig. 15 indicate that about 60 - 80% of seeds produced seedlings when the 15°~20°C cycles were applied in phase I lasting 6 or 6.5 months. Worse effects were obtained (one seed lot only tested) after phase I at 15°~25°C. When after sowing so pretreated seeds two different temperature systems were applied in phase III during the seedling emergence test i.e. 20°C and 3°~20°C, the superiority of the 15°~20°C over the 15°~25°C cyclically alternating temperature in phase I could be demonstrated (table 10). However, the temperature applied in phase III did not differentiate the percent of emerged seedlings. It should be stressed that at 20°C the seedlings emerged faster than at 3°~20°C (figs. 16 and 17). At 20° seedling emergence occured in

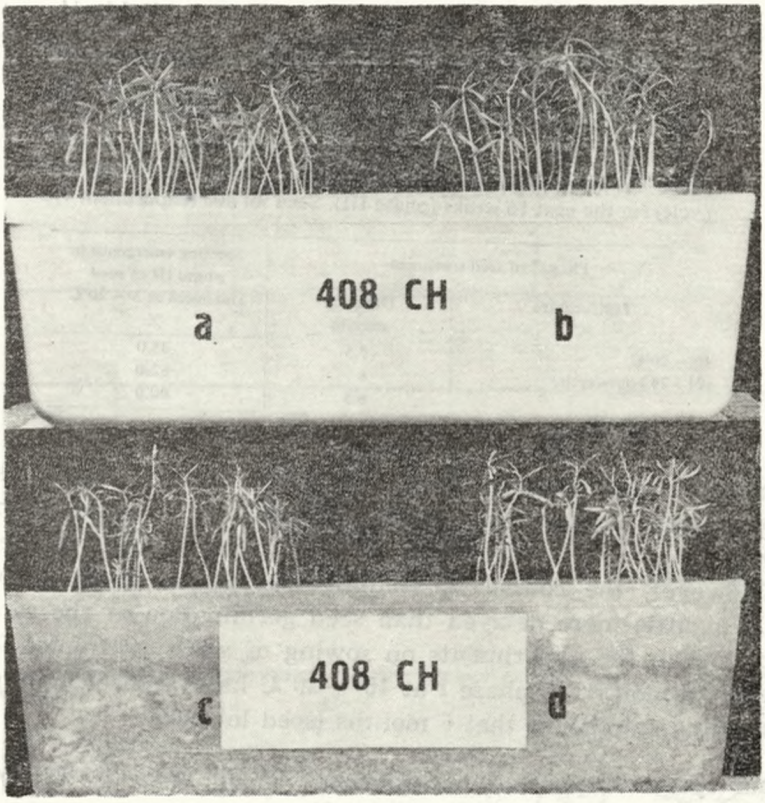

Fig. 17. Seedlings from *Taxus baccata* L. seeds (seed lot 408) in the seedling emergence test at 20°C (phase III) preceded by 6 months of stratification of seeds at 15°~20°C (48-hour cycles, 24+24 hours/cycle) followed by 4 months of stratification at 3°C (phase II). In the test 4 replicates of 50 stratified seeds were sown at a depth of 1 cm into a peat mull : sand medium and were covered with 1 cm of sand. (Experiment 7). Phot. K. Jakusz

the period between the 1st and the 4th week and was very energetic, at 3°~20°C it started 3 - 4 weeks after sowing and was terminated not earlier than after 12 - 14 weeks. This means that yew seeds properly pretreated are no more endangered by the possibility of an induction of secondary dormancy and can be sown late in spring.

In the same experiment 7 it was also investigated whether the seeds can be subjected to the thermal treatment comprising all 3 phases when the conditions for embryo growth and activation, seed after-ripening and germination are created in the sowing medium from the start, without any stratification *sensu stricto*. In fig. 18 and table 11 results are presented which indicate, that in the case of seed lot 408 so treated again about 60% of seeds have produced seedlings at 3°~20°C when 15°~20°C in phase I was acting for 6 or 6.5 months but not shorter, and when phase II at 3°C lasted 4 months. It is evident that sowing

Table 11

Seedling emergence (in %) from *Taxus baccata* L. seeds in phase III of treatment. The boxes with the sown seeds were held at first at 15°~20°C (24+24 hours/cycle) for 5½, 6 and 6½ months (phase I), after that at 3°C for 4 months (phase II), finally they were placed under cyclically alternating temperature 3°~20°C (16+8 hours/cycle) for the next 16 weeks (phase III). Seed lot 408 (experiment 7)

Phase I of seed treatment		Seedling emergence in phase III of seed treatment at 3°~20°C
Temperature °C	Duration months	%
15°~20°C (24+24 hours/cycle)	5.5	45.0
	6	62.0
	6.5	60.0

medium can completely replace stratification, when it assures simultaneously a good aeration and moisture supply, and when temperature in any phase of the treatment in always acting at the most effective level. However, the course of seedling emergence at 3°~20°C is in phase III a little more delayed than seed germination at the same temperature. As in the experiments on sowing of seeds pretreated by stratification, 6.5 months of phase I at 15°~20°C have assured a more energetic seedling emergence that 6 months (seed lot 408).

Thermal cycles in phase I of treatment longer than 1+1 day (experiment 8)

Devillez (1976, 1978) has proposed 12+12-hour cycles with 15°C in the cooler part of each cycle and 25°C in the warmer one. In our above reported work this type of temperature alternation was successfully replaced by (1+1 day) cycles basing also on 15°C but differing in the warmer stage of each cycle only by 5°C (15°~20°C). The application of the 2-day cycles (48 hours) has simplified the manipulations with the stratified or sown seeds. When one has at ones disposal only one chamber with temperature alternations programmed for 24-hour intervals or 2 chambers — each running at one of the two temperatures of the cycle — change of temperature or transfer of the seeds from one chamber to another has to be done once a day only, always at the same hour.

To simplify this system even more we have tried in experiment 8 to extend the duration of each cycle (15°~20°C) from 48 hours to 96, 192 and 384 hours. In this way both temperatures would act for 2+2, 4+4 and 8+8 days in each cycle instead of 1+1 days. Temperature alternating cyclically was acting for 6.5 months (phase I) being followed by chilling at 3°C lasting 4 months (phase II). After that the so pretreated seeds were stratified still longer or they were sown in laboratory

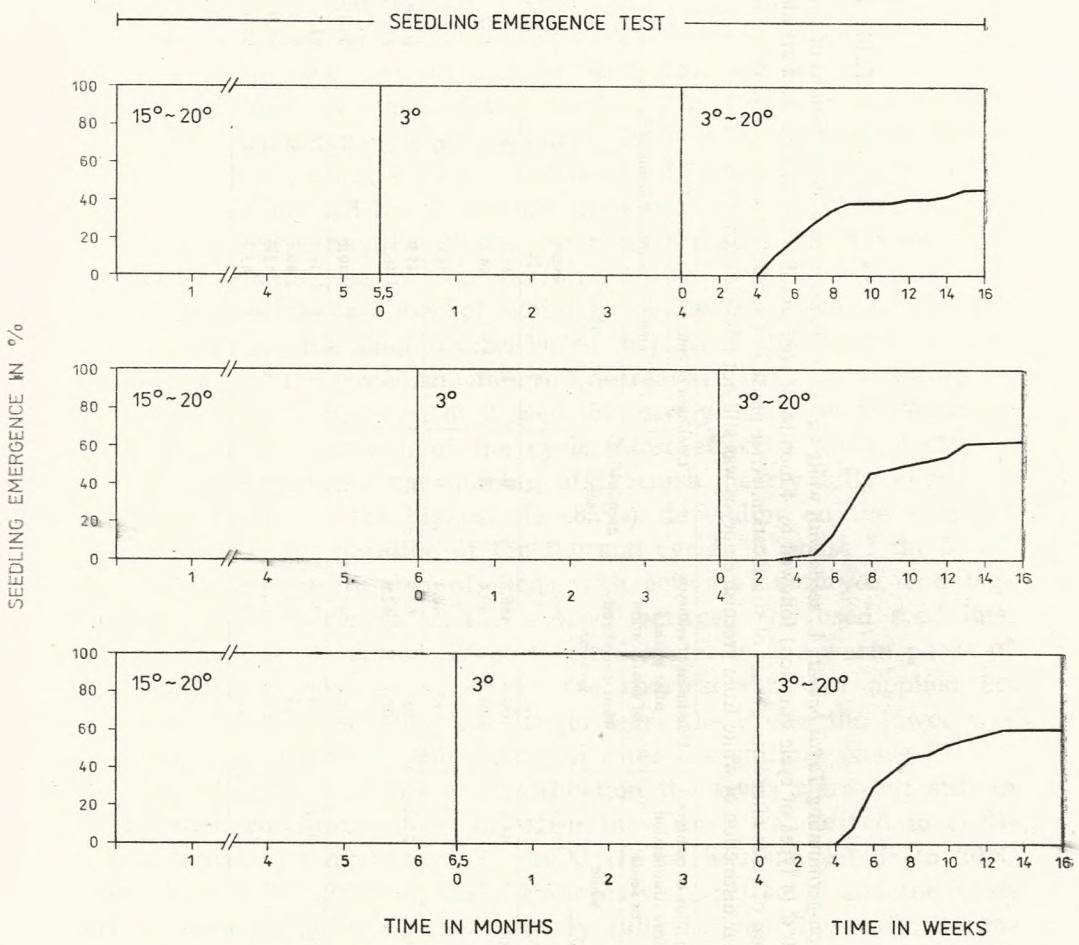

Fig. 18. Course of seedling emergence from *Taxus baccata* L. seeds (seed lot 408) during seedling emergence tests. The seeds were sown in laboratory conditions and the test boxes were subjected to a 3-phase treatment. In phase I 48-hour 15°~20°C cycles (24+24 hours/cycle) were applied, duration of that phase was differentiated. In phase III 24-hour 3°~20°C cycles (16+8 hours/cycle) were used for seedling emergence. (Experiment 7)

conditions at constant or alternating temperatures for a time permitting total germination or seedling emergence. Three different seed lots were used separately for this experiment, but only for one of them all types of the mentioned above thermal cycles could be tested. In the case of the two other seed lots one of these types (4+4 days/cycle) had to be omitted, because of insufficient amount of seeds.

For all types of thermal cycles (1+1, 2+2, 4+4, and 8+8 days) applied to the seed lots, data are presented on the development of embryos after phase I and II (table 12). These data show clearly that during stratification phase I embryos grew within the seeds though

Table 12

Number and indices of embryos (as % values) from non-germinating *Taxus baccata* L. seeds with white and greenish embryos after 6.5 months of stratification at cyclically alternating temperature 15°~20°C (4 types of cycle duration) followed by 4 months of cold stratification at 3°C. Data are also presented for non-treated dry seeds and on germination of seeds after both phases of stratification. After phase I of stratification only mean values for all seeds could be calculated, because a clear distinction of white from greenish embryos was still not possible (experiment 8)

Seed lot	Stratification phase I at 15°~20°C		Stratification phase II	Condition of embryos before stratification of seeds		Condition of embryos after phase I of stratification		Condition of embryos after phase II of stratification				
				white		white and slightly greenish		germinating seeds	embryos from non-germinating seeds			
									white		greenish	
	duration	duration of thermal cycles	duration		index		index			index		index
	months	days	months	%	%	%	%	%	%	%	%	%
449	6½	1+1	4	100	44.6	100	56.1	0	15	49.2	85	94.6
		2+2				100	49.4	0	28	44.4	72	93.2
		4+4				–	–	–	–	–	–	–
		8+8				98*	44.4	0	75	44.0	25	96.7
450	6½	1+1	4	100	37.2	98*	63.9	24	5	45.9	71	70.7
		2+2				100	62.6	36	6	46.8	58	57.6
		4+4				–	–	–	–	–	–	–
		8+8				100	49.7	12	52	40.8	36	36.1
451	6½	1+1	4	100	36.7	100	70.6	14	13	39.7	73	97.5
		2+2				100	63.5	16	32	40.6	52	97.3
		4+4				100	60.6	12	35	42.6	53	99.9
		8+8				100	56.5	6	52	43.3	42	93.9

* the remaining 2% of seeds decayed

their greening could not be easily observed in this period. However, the longer the cycles lasted the lower were the values of the embryo index. In seeds from seed lot 449 subjected to the longest cycles (8+8 days) the embryos did not grow at all. The seed lots used for experiment 8 differed somewhat in their reaction to the applied type of the thermal cycle. Seeds from lots 450 and 451 have germinated during the last 2 weeks of chilling (phase II) by 12 - 36% and 6 - 16% respectively, while those from lot 449 did not even start germination while chilled for the previously fixed 4 months. When the measurements of embryos from the non-germinating seeds were performed after both (I and II) phases of stratification the number of white non-activated embryos was the bigger the longer the single cycles lasted in phase I, and simultaneously the number of the greenish embryos decreased. The index values of the latter were in the case of 2 seed lots always close to 100%, independently of the duration of the cycle. Most effective were cycles of the 1+1-day type, here the number of greenish, nearly fully elongated embryos was always the highest (71 - 85%), depending on the seed lot. The longer was the duration of the thermal cycles in phase I the lower was the percentage number of seeds with activated embryos, and bigger were the differences in this respect between the used seed lots. We can conclude: extension of the thermal cycles in the warm phase of stratification of yew seeds beyond the shortest duration applied i.e. 1+1 days is not reasonable. The longer were the cycles the lower was the number of activated embryos, even after the chilling phase.

When after both phases of stratification the seeds were left still in stratification conditions at 3° or when they were transferred to cyclically alternating temperature 3°~20°C (16+8 hours/cycle) or to 20°C most of the slowly growing white embryos were activated and the seeds germinated together with those already fully active. This could be observed in all variants independently of the temperature, though the speed of germination was differentiated.

In general germination after termination of stratification (phases I and II) occured in the following time intervals:

at 3°C from the 0 - 2nd week till the 8 - 10th week
at 3°~20°C from the 0 - 1st week till the 2 - 4th week
at 20°C within the first 2 weeks

In this way we can expect the mass germination to be completed after stratification at the latest after 8 weeks at 3°C, after 3 weeks at 3°~20°C and after 2 weeks at 20°C.

Seedling emergence after sowing of stratified seeds started later and lasted longer:

at 3°~20°C from the 2 - 4th week till the 10 - 12th week
at 20°C from the 0 - 3rd week till the 4 - 9th week.

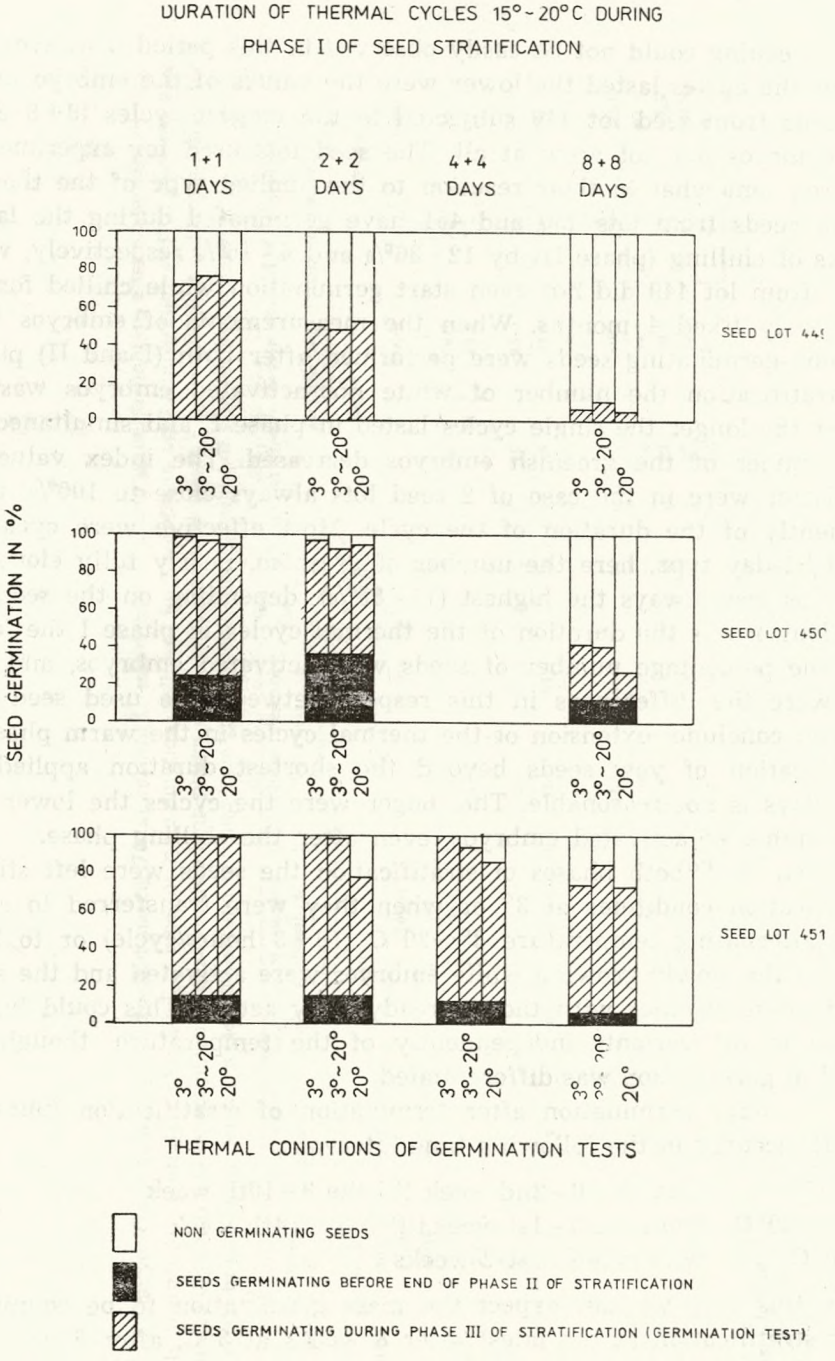

Fig. 19. Final result of germination tests of *Taxus baccata* L. seeds (seed lots 449, 450 and 451). In phase I the seeds were stratified for 6.5 months at 15°~20°C but 4 types of thermal cycles were applied in the individual experimental series: 1+1 days, 2+2 days, 4+4 days and 8+8 days. During phase II stratification was continued at 3°C for 4 months. In phase III temperature of stratification was differentiated (3°, 20°C and 3°~20°C, 16+8 hours/cycle). (Experiment 8)

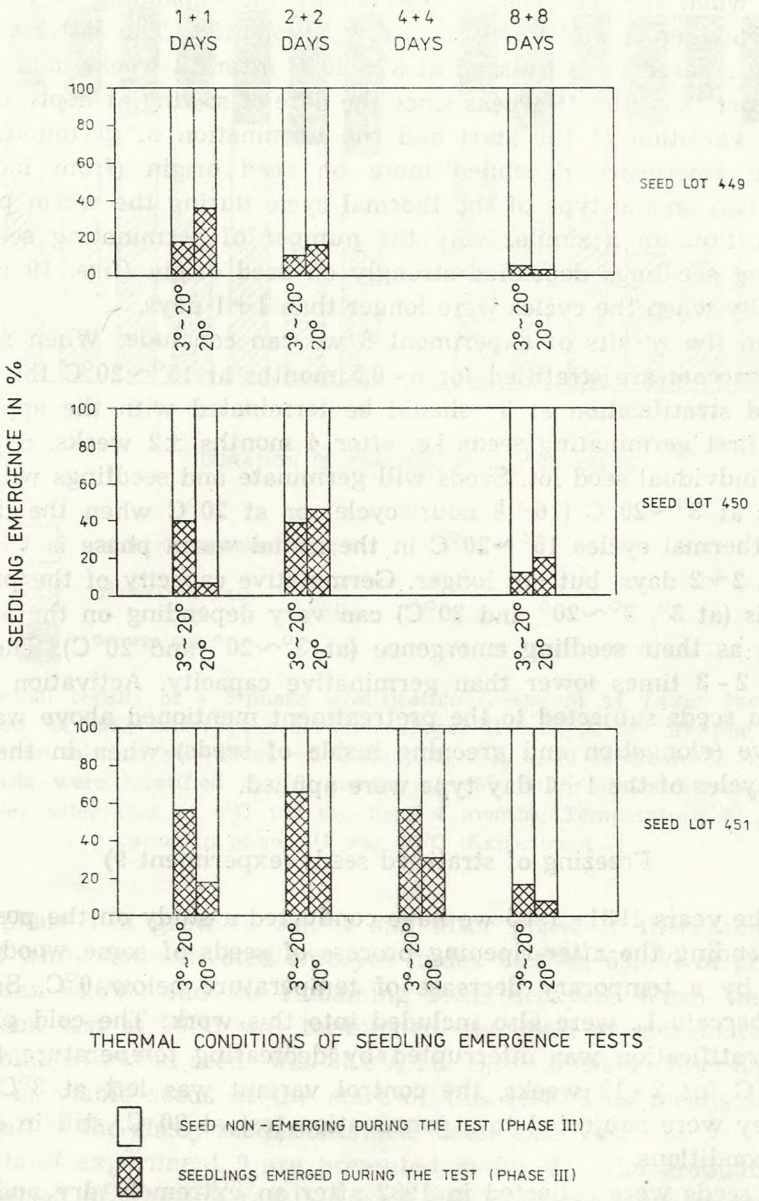

Fig. 20. Final results of seedling emergence tests of *Taxus baccata* L. seeds (seed lots 449, 450 and 451). The tests (phase III) were preceded by stratification at 15°~20°C lasting 6.5 months (phase I) followed by 4 months of stratification at 3°C. In phase I 4 types of thermal cycles were applied in the individual experimental series: 1+1 days, 2+2 days, 4+4 days and 8+8 days. During phase II stratification was continued for 4 months at 3°C, after that the seeds were sown in laboratory conditions into a peat mull : sand medium at a depth of 1 cm and were covered with 1 cm of sand. In the seedling emergence tests (phase III) temperature was differentiated (20° or 3°~20°C, 16+8 hours/cycle). (Experiment 8)

Seedling emergence starts with the appearance of the bowshaped hypocotyl above the surface of the sowing medium and is finished completely when the seed coat is rejected by the expanding cotyledons of the already erect and vertically growing plantlet. The latter phase of seedling emergence is finished at 3°~20°C after 12 weeks, and at 20°C not earlier than after 9 weeks since the date of sowing at depth of 1 cm.

The variation of the start and the termination of germination and seedling emergence depended more on seed origin (from individual trees) than on the type of the thermal cycle during the warm phase of stratification. In a similar way the number of germinating seeds and emerging seedlings depended strongly on seed origin (figs. 19 and 20), especially when the cycles were longer than 1+1 days.

From the results of experiment 8 we can conclude: When seeds of *Taxus baccata* are stratified for 6 - 6.5 months at 15°~20°C the following cold stratification at 3° should be terminated with the appearance of the first germinating seeds i.e. after 4 months ±2 weeks, depending on the indvidual seed lot. Seeds will germinate and seedlings will emerge best at 3°~20°C (16+8 hours/cycle) or at 20°C when the duration of the thermal cycles 15°~20°C in the initial warm phase is 1+1 days or even 2+2 days, but not longer. Germinative capacity of the pretreated seeds (at 3°, 3°~20° and 20°C) can vary depending on the seed lot, as well as their seedling emergence (at 3°~20° and 20°C). The latter can be 2 - 3 times lower than germinative capacity. Activation of embryos in seeds subjected to the pretreatment mentioned above was most intensive (elongation and greening inside of seeds) when in the warm phase cycles of the 1+1 day type were applied.

Freezing of stratified seeds (experiment 9)

In the years 1981 - 1985 we have conducted a study on the possibility of suspending the after-ripening process of seeds of some woody plant species by a temporary decrease of temperature below 0°C. Seeds of *Taxus baccata* L. were also included into this work. The cold phase of their stratification was interrupted by decreasing temperature to −1° or −3°C for 2 - 12 weeks, the control variant was left at 3°C. After that they were subjected to a germination test at 20°C, still in stratification conditions.

The seeds were collected in 1982 after an extremely dry and warm summer. Though they were filled 100.0% part of them (52.0%) had a glassy consistency and it was very difficult to distinguish the embryos. However after phase I and II of stratification the embryos became clearly visible, though after phase I it was still not possible to distinguish the white embryos from the slightly greenish ones.

At the start of the experiment the mean embryo index was 44.2%.

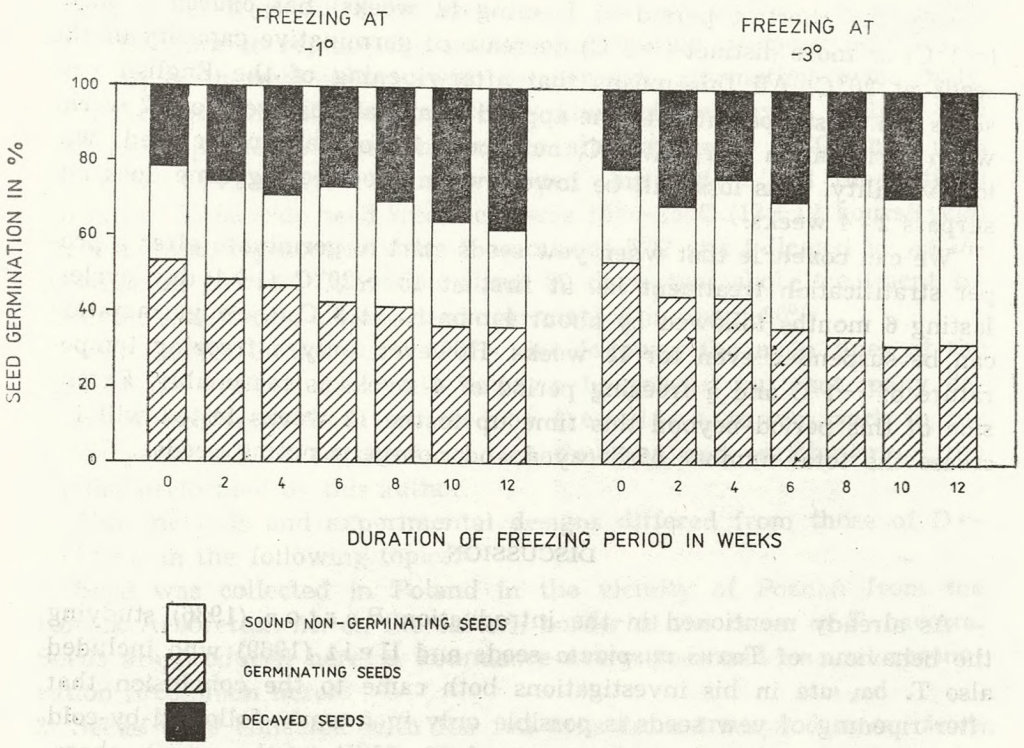

Fig. 21. Final results of a 3-phase stratification treatment of *Taxus baccata* L. seeds (seed lot 559), interrupted between phases II and III by freezing of the seed : medium mixture at −1° or −3°C for 0, 2, 4, 6, 8, 10 or 12 weeks. In phase I the seeds were stratified for 6 months at 15°~20°C (48-hours/cycle, 24+24 hours/cycle), after that at 3°C for the next 4 months. Temperature of stratification in phase III was 20°C (Experiment 9)

During phase I it grew to 54.0% and after phase II there remained 17.5% of white non-activated embryos (index 33.8%), 65.0% of greenish ones (index 68.6%) and the remaining seeds decayed. When the final germination test at 20°C was terminated, the total of germinated and non-germinated sound seeds was 88.5% i.e. close to the 17.7%+65.0%= =82.0% of viable seeds at the start of this test. This means that at least a half of the glassy seeds contained viable embryos.

Results of experiment 9 are presented in fig. 21. The gradually extended period of freezing resulted in a steady decrease of the number of germinating seeds from 53.5% in the control variant (no freezing) down to 36.0% and 32.0% for −1° and −3°C respectively. When the cold break was performed at −3°C the percent of germinating seeds was always somewhat lower than when it was done at −1°C. The remaining seeds could be divided nearly half by half into non-germinating sound seeds and the decaying ones. Independently of the temperature

already the shortest period of freezing (2 weeks) has caused a slight (−1°C) or more distinct (−3°C) decrease of germinative capacity of the seeds at 20°C. All this means that after-ripening of the English yew seeds can be stopped at both the applied temperatures even for 12 weeks when germination starts at 3°C, but about 1/3 of germinable seeds will lose viability. This loss will be lowest when the freezing time does not surpass 2 - 4 weeks.

We can conclude that when yew seeds start to germinate after a proper stratification treatment i.e. at first at 15°~20°C (1+1 day cycles) lasting 6 months followed by about 4 months at 3°C, their germination can be suspended even for 12 weeks. However, only a freezing temperature of −1°C and a freezing period of 4 weeks is reasonable. Extension of this period beyond this time up to the 12 weeks tested will increase the total number of decayed and non-germinating seeds.

DISCUSSION

As already mentioned in the introduction Barton (1936) studying the behaviour of *Taxus cuspidata* seeds and Heit (1969) who included also *T. baccata* in his investigations both came to the conclusion that after-ripening of yew seeds is possible only in a warm-followed-by-cold thermal system with a temperature of 20 - 25°C in the warm phase. Devillez (1976, 1978) was the first to show that this warm phase should last 6 months and be performed at a daily alternating temperature in the range of 15°~25°C (12+12 hours/cycle). After termination of the next cool period at 5°C lasting 3 months germination of seeds could be triggered by incubation in another cyclically alternating temperature 10°~20°C (12+12 hours/cycle).

In the first part of the study reported here on the after-ripening and germination of *T. baccata* seeds conducted in the years 1966 - 1978 an attempt was made to use various combinations of warm and cold periods of different duration and at differentiated constant temperature levels. All these variants were unsuccessful though the longest duration of the warm phase has reached in some experiments 24 weeks, i.e. nearly 6 months. The highest germinability ever obtained was only 8.5%. However it became evident that germination of *T. baccata* seeds can be expected only when a succesion is assured of a warm period followed by a cool one and then by a warm one again. Thus germination starts early in this third, warm phase of the treatment. Scarification of seeds with concentrated sulphuric acid as well as placing them in stratification conditions at temperatures up to 40°C for a short period before cold stratification proved ineffective.

A full success i.e. energetic mass germination of seeds has been ob-

tained in our work since the adoption of the idea of Devillez (1976) from Belgium to replace the initial warm period of moist seed treatment by a thermal system with temperature alternating twice daily, with the optimum at the basal temperature of each cycle of 15°C and the the amplitude of the temperature alternations of 10°C. In a next paper Devillez (1978) has developed this system and the optimal variant of *T. baccata* seed treatment was 15°~25°C (12+12 hours/cycle) for 6 months, supplemented by 3 months at 5°C and followed by an incubation at 10°~20°C, lasting about 20 days, the whole treatment lasting 9.5 months. So treated seeds germinated in about 80%.

Our studies in the years 1977 - 1984 followed the main lines of the idea of Devillez. It should however be pointed out that many details differed from those reported by Devillez, so our work in the latter years can be regarded as a continuation and enrichment of the studies performed by this author.

Our methods and experimental designs differed from those of Devillez in the following topics:

1. Seed was collected in Poland in the vicinity of Poznań from the Kórnik Arboretum i.e. on the eastern border of the range of *T. baccata*. Seeds are produced here in abundance every year and natural regeneration is common here.
2. Seeds were collected with the red arils from trees and never from the ground.
3. Seeds were always stratified in a moist sand : peat mull medium, but never on moist filter paper nor in the surface layer of sand.
4. In some experimental variants the pretreated seeds were sown in laboratory conditions in the medium as above, and only seedling emergence was observed in such cases.
5. In one experiment seeds were also sown from the very start of the treatment in boxes with the standard medium enriched with macro- and microelements from the Azofoska mineral nutrient and all thermal phases (I, II and III) were run in these sowing conditions. Here also only seedling emergence could be observed.
6. In phase I many more constant and alternating temperature variants were tested.
7. In phase I thermal cycles of the 24+24 hours (1+1 day) type were applied instead of the 12+12 hours type of Devillez, and in one experiment also cycles of 2+2, 4+4 and 8+8 days type were tried.
8. Some experiments were conducted on seeds collected separately from individual trees.
9. Phase II (the cold one) was conducted at 3°C and not at 5°C.
10. Phase III (incubation of Devillez) was tested at 3°C, 20°C and the alternating temperature 3°~20°C (16+8 hours/cycle) and not at 10°~20°C (12+12 hours/cycle) as in the work of Devillez.

11. Growth of embryos in phase I and II of treatment was expressed as % of the total length of the female gametophyte (called improperly "endosperm"). The necessary data were obtained by direct measuring of the gamethophyte and of the izolated embryos but not of the length only of the embryo as in the X-ray method of Devillez.

Taking these differences into account results were obtained confirming those of Devillez or adding new informations to the knowledge of seed behaviour of *T. baccata*. It was shown for yew seed of Polish origin that thermal cycles of the 15°~20°C type are more effective than those of the 15°~25°C type, i.e. that the smaller amplitude of 5°C between both temperature levels of each cycle is more efficient. To simplify the operations only the 24+24 hours cycles were applied and such temperature alternations have assured results not worse than those obtained with the 12+12 hours/cycle by Devillez. The duration of both components of each cycle can be extended even to a 8+8 days/cycle but already the still successful variant of 2+2 days has contributed to a decrease of germinability in phase III. A further extension of the cycles over 2+2 days is no more advisable, because of too severe losses in seed viability. It was also shown that the necessary duration of phase I is variable, some seeds collected in the same place but from other trees need a rather longer treatment which should be prolonged to 6.5 months. We have also decided to run cold phase II of seed treatment until the first germinating seeds indicate that some seeds at least have satisfied their temperature requirements. This period was in the case of the tested seeds never shorter than 4 months, sometimes it was even a little longer. When so pretreated seeds were transferred or sown at 3°C their germination started late and was slow, at 20°C like in the experiments of Devillez it started nearly immediately and lasted not longer than 2 weeks, at 3°~20°C it started only after a short lag and was terminated latest after 4 weeks of this phase. When only seedling emergence could be observed (after sowing) and the sown seeds produced seedlings, this process was faster and earlier at 20°C than at 3°~20°C. These results are so important because in the nursery germination is only the first step to seedling emergence i.e. the transformation of seeds into plantlets.

As the result of our studies on *Taxus baccata* seeds we can recommend the following 3-phase treatment:

phase I 15°~20°C (24+24 hours/cycle) for 6 - 6.5 months
phase II 3° for 4 - 4.5 months until the seeds start to germinate
phase III 3°~20°C (16+8 hours/cycle)

The seeds can be either pretreated by stratification in phases I and II, after which they can be sown in the spring, or they can be sown from the very beginning and the boxes with the sown seeds can be placed successively in appropriate thermal conditions. The start of the

treatment should make possible a direct transfer from phase II to III in the spring. When weather conditions make direct sowing impossible and the seeds start to germinate, containers with the stratified seeds (or boxes with the sown seeds) can be held for a relatively short time (2 - 4 weeks) at -1° to -3°C. This prevents them from germination. After defrosting the medium these seeds can be sown or the boxes transferred and only a small proportion will be harmed.

In 1982 and 1983 seeds were collected after extremely warm and dry summers and autumns. In 1983 many seeds did not develop the firm and white female gametophyte tissue, which was glassy and when exposed has very early lost ist bright colouration. Even such seeds did germinate and produce seedlings, though the latter were much more susceptible to damping off than those from seeds collected in the other, more normal years, especially when the seeds were sown after pretreatment or treated by various temperatures since the very start in the sown condition.

The problem of the behaviour of yew seeds in natural conditions has some importance in the discussion on the protection of *T. baccata* in our forests — where as a species it is close to extinction. It seems, that yew can regenerate sexually only where seeds fallen to the ground in the autumn are proctected againts drying out, are placed by accident or by the action of animals or birds in the moist upper layers of the soil but only in such places, where daily temperature alternations in the range between $15^{\circ}\sim20^{\circ}$C and $15^{\circ}\sim25^{\circ}$C are assured over a long period between the late spring and early autumn. When in winter or at least in late autumn and early spring of next year temperature around the seeds remains at the 0 - 5°C level for at least 3, or even better for 4 months, abundant germination and seedling emergence in spring can be expected, when the other conditions for germination and seedling emergence will be provided.

We cannot agree completely with D e v i l l e z (1978) that the initial warm phase of seed treatment does not contribute to the morphological ripening of the embryos within the seeds. We could observe, e.g. in experiment 6 (table 1) that in the optimal thermal conditions the majority of embryos (85% at $15^{\circ}\sim20^{\circ}$C and 90% at $15^{\circ}\sim25^{\circ}$C became greenish and grew so that their index was 70.2% and 61.7% respectively. This means that their length was nearly doubled only during the warm phase, when it lasted 6 - 6.5 months. In other experimental series the mean embryo index of the greenish embryos was either greater or lower than that mentioned above (e.g. table 4). D e v i l l e z could not observe the appearance of the greenish embryos and their much faster growth in contrast to the white ones, because of the X-ray method he used. The activated embryos continue their growth in the cold phase when their after-ripening occurs, simultaneously, and this growth is

enhanced after increase of temperature in phase III, resulting in germination. The higher temperature (20°C in that phase acting at least for a part (1/3) of every day is necessary not only for the growth of the radicle possible also at low temperature, but in particular for the elongation of the hypocotyl, an essential condition for seedling emergence.

In regions where the climate makes the long lasting moderately warm and gently alternating temperature in the upper soil layer in shady places in summer impossible and where in winter the soil is frozen and the transitions from autumn to winter and from winter to spring are rapid or short lasting like in continentally influenced climatic regions, the necessary growth and activation of embryos within the seeds in summer and the continuation of this growth and their germination in the colder half of the year are not possible. We cannot except in such years or in places with such climate any natural regeneration of *Taxus baccata*. The same conditions are also limiting the natural range of this species. Changes of the climate at bigger time intervals and changes in the natural environment including the extinction of birds responsible for the removal of the seeds from below the mother trees or from the trees themselves and for their transport contributes also to the cessation of the natural sexual regeneration of this species. Experimental work has helped to recognize the requirements of yew seeds making their imitation in controlled conditions possible.

SUMMARY

When *Taxus baccata* L. seeds of Polish origin, collected when the arils were red and the seed surface dark, fresh or dry-cold-stored, were used for investigations, conditions for the activation and growth of embryos within the seeds, for their after-ripening, for germination and for seedling emergence were created by subjecting them to the following 3-phase treatment:

[phase I] stratification at 15°~20°C (24+24 hours/cycle) 6.5 months

+

[phase II] stratification at 3°C 4 - 4.5 months, until the first seeds start to germinate

+

[phase III] 20°C 2 weeks for germination, 4 - 9 weeks for total seedling emergence

or

3°~20°C (16+8 hours/cycle) 2 - 4 weeks for germination
10 - 12 weeks for total seedling emergence

In this way the total duration of the pretreatment preceding the date of sowing (phases I+II) is 10 - 11 months.

Application of constant temperature in phase I makes further germination of seeds impossible or it reduces it to a very low level. At the cyclically alternating temperature in phase I growth and greening of embryos are initiated, being continued in the cool phase II, when simultaneously after-ripening of the seeds occurs and dormancy disappears. Germination becomes possible even at the low temperature of phase II when the latter is continued, but higher temperature or a cyclically alternating one in phase III makes an early start of germination possible.

The proposed treatment can be applied both to freshly collected seeds and to seeds stored in a dried condition (10% of moisture content) at low temperature (e.g. $-3°C$) in sealed containers.

Seed lots from individual trees even when collected on the same site in the same season can differ in the necessary duration of phases I and II of the pretreatment, so the cool phase II should be not terminated until the first seeds start to germinate. Phase I can be extended up to 6.5 months.

It is advisable to start pretreatment of *T. baccata* seed 10 - 11 months before the planned date of sowing in spring. When weather conditions (frost, rain, drought) make sowing of seeds still impossible germination can be stopped by placing the stratified seeds together with the medium at $-1°$ to $-3°C$ for a period not longer than 2 - 4 weeks. After defrosting of the medium the seeds will germinate when sown and their germinability will be only slightly lower. This decrease of germinability caused by decaying of seeds is greater the longer the seeds are held in a frozen medium.

LITERATURE

1. Anonim, 1948. Woody-Plant Seed Manual. U.S. Dep. of Agric., Misc. Publ. No. 654. Washington D.C.
2. Bartkowiak S., 1970. Ornitochoria rodzimych i obcych gatunków drzew i krzewów. Arboretum Kórnickie 15: 237 - 261.
3. Bartkowiak S., Zieliński J., 1973. Rola synzoochrii w naturalnym odnowieniu cisa (*Taxus baccata* L.). Arboretum Kórnickie 18 : 265 - 272.
4. Barton L. V., 1939. Experiments at Boyce Thompson Institute on germination and dormancy in seeds. Scientific Hort. Bull. 7 : 186 - 193.
5. Devillez F., 1976. Afterripening and dormancy breakdown in *Taxus baccata* L. seeds. IUFRO S2.01.06. Proc. 2nd Interntl. Symposium "Physiology of seed germination". Fuji, Japan, 1976.
6. Devillez F., 1978. Influence de la température sur la postmaturation et la germination des graines de l'if (*Taxus baccata* L.). Acad. Royale de Belgique, Bulletin de la Classe des Sciences 5e série — Tome LXIV 1978-4, 203 - 218.

7. Heit C. E., 1967. Propagation from seed — Part 10. Storage methods for conifer seeds. Amer. Nurseryman 126(8) : 4 - 15, 38 - 54.
8. Heit C. E., 1969. Propagation from seed — Part 18. Testing and growing seeds of popular *Taxus* forms. Amer. Nurseryman 129(2):10 - 11, 118 - 128.
9. Jackowski J., 1972. Naturalne odnowienie cisa na Pomorzu Zachodnim. Sylwan 116(11):47 - 52.
10. Janson L., 1975. Hodowla, rozmnażanie i uprawa cisa. Las Polski 49(18):13 - 15.
11. Król S., 1978. An outline of ecology. In: The Yew — *Taxus baccata* L. (Cis pospolity — *Taxus baccata* L.). Nat. Center for Scientific, Technical and Economic Information, Warsaw, 65 - 86.
12. Krüssmann G., 1964. Die Baumschule. Verlag Paul Parey, Berlin u. Hamburg.
13. Lamb J. G. D., Kelly J. C., Bowbrick P., 1975. Nursery stock manual. Grower Books, London.
14. Le Page-Degivry M.-Th., 1973a. Étude en culture in vitro de la dormance embryonnaire chez *Taxus baccata* L. Biologia Plantarum, 15 : 264 - 269.
15. Le Page-Degivry M.-Th., 1973b. Intervention d'un inhibiteur lié dans la dormance embryonnaire de *Taxus baccata* L. C.R. Acad. Sc. Paris, 177:177 - 180.
16. Le Page-Degivry M.-Th., Garello G., 1973. La dormance embryonnaire chez *Taxus baccata* L.: influence de la composition du milieu liquide sur l'induction de la germination. Physiologia Plantarum 29:204 - 207.
17. Rudolf P. O., 1974. *Taxus* L. Yew. In: Seeds of Woody Plants in the United States. For. Service, USDA, Agriculture Handbook No. 450. Washington D.C. Pp. 799 - 802.
18. Suszka B., 1978. Generative and vegetative reproduction. In: The Yew — *Taxus baccata* L. (Cis pospolity — *Taxus baccata* L.). Nat. Center for Scientific, Technical and Economic Information, Warsaw. 87 - 102.
19. Tyszkiewicz S., 1949. Nasiennictwo Leśne. Instytut Badawczy Leśnictwa, Seria D, Nr 2. Warszawa. 272 - 273.
20. Tyszkiewicz S., Dąbrowska J., 1953. Stratyfikacja nasion drzew i krzewów leśnych. Roczn. Nauk.-Leśn. 1:155 - 221.
21. Wappes L., 1932. Wald u. Holz, ein Nachschlagebuch für die Praxis der Forstwirte, Holzhändler und Holzindustriellen, I Band. Neudamm. P. 257.
22. Zenkteller M. A., Guzowska J., 1970. Cytological studies on the regenerating mature female gametophyte of *Taxus baccata* L. and mature endosperm of *Tilia platyphyllos* Scop. in in vitro culture. Acta Soc. Bot. Pol. 39(1):161 - 173.

Warunki ustępowania spoczynku, kiełkowania i wschodzenia nasion cisa pospolitego (*Taxusc bacata* L.)

Streszczenie

Nasiona cisa pospolitego (*Taxus baccata* L.) zbierano w Polsce, w Arboretum Kórnickim, w stanie dojrzałym po przybraniu przez osnówki barwy czerwonej i używano po oczyszczeniu do badań w stanie świeżym lub po przechowaniu w chłodni w stanie podsuszonym. Badania dotyczyły ustępowania spoczynku tych nasion. Aktywację i wzrost zarodków w nasionach, kiełkowanie nasion i ich wschodzenie po wysiewie umożliwiało poddanie nasion traktowaniu rozkładającemu się na 3 fazy:

[faza I] stratyfikacja w 15°~20°C 24+24 godz./cykl przez 6 - 6,5 miesiąca

+

[faza II] stratyfikacja w 3°C przez 4 - 4,5 miesiąca, do pojawienia się pierwszych nasion kiełkujących

+

[faza III] w 20°C przez 2 tygodnie (kiełkowanie) lub 4 - 9 tygodni (wschody)

albo

w 3°~20°C 16+8 godz./cykl przez 2 - 4 tygodni (kiełkowanie) lub 10 - 12 tygodni (wschody)

Przedsiewne traktowanie nasion cisa (fazy I+II) trwa więc łącznie 10 - 11 miesięcy.

Temperatura stała w fazie I uniemożliwia późniejsze kiełkowanie lub redukuje zdolność kiełkowania nasion do bardzo niskiego poziomu. W temperaturze cyklicznie zmiennej w fazie I zarodki znajdujące się w nasionach zaczynają się zazieleniać i rosnąć. Proces ten jest kontynuowany w chłodnej fazie II stratyfikacji lub traktowania nasion innym sposobem (np. po wysiewie). Wtedy to dopiero ustępuje spoczynek nasion, a ich kiełkowanie staje się możliwe, nawet w warunkach chłodnej stratyfikacji, jeżeli ta nie zostanie przerwana. W temperaturze wyższej lub cyklicznie zmiennej w fazie III kiełkowanie rozpoczyna się wcześnie i przebiega szybko.

Proponowany tu sposób traktowania nasion cisa może być zastosowany zarówno dla nasion świeżo pozyskanych i oczyszczonych, jak i dla nasion przechowywanych w stanie podsuszonym (10% zawartości wody) w chłodni (np. w −3°C) w szczelnie zamkniętych pojemnikach.

Nasiona pozyskane w tym samym sezonie z indywidualnych drzew rosnących na tym samym stanowisku mogą się różnić pod względem długości fazy I i II. Z tego powodu fazę II należy kontynuować aż do pojawienia się pierwszych nasion kiełkujących, co następuje po 4 - 4,5 miesiącach, faza I może być przedłużona do 6,5 miesięcy.

Zaleca się rozpoczęcie przedsiewnego traktowania nasion cisa pospolitego na 10 - 11 miesięcy przed planowaną datą siewu wiosennego. Jeżeli niekorzystne warunki pogody (mróz, opady, susza) uniemożliwiają siewy, można kiełkowanie powstrzymać przez umieszczenie nasion wraz z podłożem stratyfikacyjnym w chłodni w temperaturze od −1° do −3°C na okres nie dłuższy niż 2 - 4 tygodnie. Nasiona wysiane po rozmrożeniu podłoża kiełkują w procencie nieznacznie niższym niż nasiona niemrożone. Spadek zdolności kiełkowania jest następstwem zamierania części nasion i jest tym większy, im dłużej nasiona przebywają w zamrożonym podłożu.

Условия уступания состояния покоя, прорастания и появления всходов семян тисса ягодного (*Taxus baccata* L.)*

Резюме

Семена тисса ягодного (*Taxus baccata* L.) собирали в Курникском Арборетуме, в Польше, в зрелом состоянии после приобретения кровелькой красного цвета. Для опытов их употребляли в свежем виде после очистки или после хранения в холодильных камерах в подсушенном состоянии. Темой исследования было уступание состо-

* Автор Б. Сушка.

яния покоя этих семян. Активация и рост зародышей в семенах, прорастание семян и появление всходов после посева было возможным после их обработки состоящей из 3 фаз:

фаза I стратификация при 15~20°C (24+24 часов/цикл) продолжительностью 6 - 6,5 месяца

+

фаза II стратификация при 3°C в течение 4 - 4,5 месяцев, до момента появления первых проросших семян

+

фаза III длительность ее 2 недели при 20°C (прорастание) или 4 - 9 недель (появление всходов)

или

при 3~20°C (16+8 часов/цикл продолжительностью 2 - 4 недели (прорастание) или 10 - 12 недель (появление всходов)

Таким образом представленная обработка семян тисса (фазы I+II) длится в общей сложности 10 - 11 месяцев.

Постоянная температура в фазе I препятствует прорастанию семян в более позднее время или ограничивает способность прорастания семян до очень низкого уровня. При циклических изменениях температуры в фазе I находящиеся в семенах зародыши начинают зеленеть и расти. Этот процесс продолжается в холодной фазе II стратификации или во время обработки семян другим способом (например после высева). Лишь тогда только уступает состояние покоя семян. а их прорастание становится возможным, даже при условиях холодной стратификации, если ее не прекратить При более высокой температуре или ее циклических изменениях в фазе III прорастание начинается раньше и протекает быстрее.

Предложенный способ обработки семян тисса можно применять как для свежесобранных и очищенных семян тисса, так и для семян хранимых в подсушенном состоянии (10% содержания воды) в холодильных камерах (например при −3°C) в плотно закрытых контейнерах.

Семена собранные в тот же период с отдельных деревьев растущих в схожих условиях могут отличаться продолжительностью I и II фазы. Ввиду этого фазу II необходимо продолжать до момента появления первых прорастающих семян, что имеет место после 4 - 4,5 месяцев. Фазу I можно продлить до 6,5 месяца.

Рекомендуется начать предпосевную обработку семян тисса ягодного на 10 - 11 месяцев до планируемого весеннего посева. Если неблагоприятные погодные условия (мороз, осадки, засуха) препятсвуют посеву, можно прорастание приостановить путем помещения семян вместе со стратификационным субстратом в холодильных камерах при температуре от −1° до −3°C сроком не более чем 2 - 4 недели. Семена высеянные после размораживания субстрата прорастают в значительно меньшем проценте, чем семена неподвергнутые замораживанию. Понижение способности прорастания является следствием отмирания части семян и оно тем больше, чем длительнее семена хранятся в замороженном субстрате.