Pre-sowing treatment of tree species

PRE-SOWING TREATMENT

= all procedures applied to seed lots to improve their biological and technical qualities

Pre-sowing treatment objectives:

- **1. overcoming the dormant stage**
- 2. removal of unproductive seeds
- 3. improving seed vitality
- 4. elimination of surface and internal mycoflora
- 5. improving the sowing properties of seeds

1. Overcoming the dormant stage (dormancy)

Seed germination

= the process of embryo growth restoration in a seed separated from the parent plant and embryo development into an independent seedling

Conditions for germination:

- external
 - humidity
 - oxygen
 - temperature
 - light
- internal
 - the seed is alive
 - the seed has transitioned from morphological

to physiological maturity

Seed maturation (seed maturity stages)

Milky maturity

- Waxy maturity
- Morphological maturity the seed is separating from the parent plant
- Physiological maturity the seed can germinate
 - **spruce**, **pine**, **larch**, alder, birch... enter physiological maturity with a transition into morphological maturity (may germinate)
 - fir, Douglas-fir, yew-tree (*Taxus*), beech, ash, maple... enter the dormant stage (dormancy) with a transition into morphological maturity (shedding in early autumn)

Dormancy = state where live seeds do not germinate even under the right germination conditions



- self-adjustment to seasonal and random changes of the environment
- prevents the seed from germinating at the wrong time
- increases the species' chance of survival

The fundamental causes of dormancy

seed coats

 the seed is morphologically and anatomically developed, seed coat (testa) or pericarp prevent germination

- water, gas, mechanical impermeability radicle barrier...
- Faboideae (acacia, sophora, gleditsia...), sumac, rose...

presence of growth inhibitors

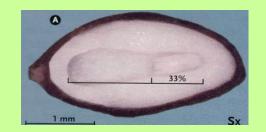
- inhibitors = ABA, org. acids, terpenes...
- in different seed structures

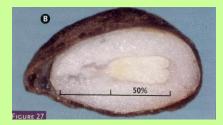
Tree species	Inhibitor placement
red oak	cotyledon
Fagus sylvatica	embryo
Corylus avellana	embryo
Abies alba	terpenes
Betula pendula	pericarp
Fraxinus excelsior	embryo, testa, endosperm
drupes	generally in the endosperm
berries	generally in the mesocarp

- maple, **beech**, birch, **Douglas-fir**, **fir**, red oak...

- morphological embryo condition
- differentiated embryo, but not reaching the entire length of the embryonal cavity







- ash, yew-tree (*Taxus*), alpine pine (*Pinus cembra*), ginkgo, holly (*Ilex sp.*)

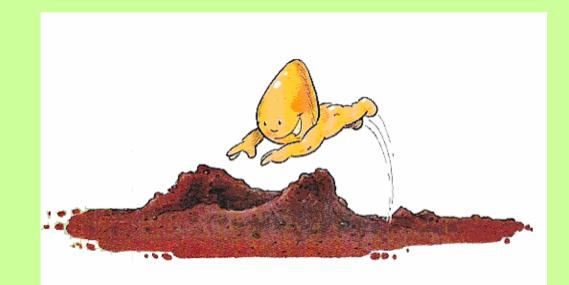


- acting alone or in combination

Overcoming dormancy in nurseries

simulation of natural conditions and their standardization

 pre-sowing treatment according to the cause of dormancy



- 1.1 Overcoming dormancy caused by seed coats Objective: disrupt seed coat (testa) or pericarp
- **1.1.1 Dry methods of seed coat disruption**
 - scarification mechanical disruption of seed coats
 - scarification equipment (scarifiers)



- scarification drums
- shaking with glass pieces
- burning needles or lamps

advantages:

the seed is dry (mechanized sowing)

disadvantages:

not for seeds with flesh the treatment period must be pre-determined the treated seeds cannot be stored

exposure to alternating temperatures volume change – seed coat rupture, laboratory use only

1.1.2 Wet methods of seed coat disruption

maceration (acidic scarification) = seed coat disruption with concentrated sulphuric acid (H₂SO₄)

Equipment:

- 95% H₂SO₄
- acid resistant container
- rinsing screens
- water supply
- protective equipment + trained personnel

procedure:

- temperate the seeds
- dip into acid for a given time (acid : seeds = 2 : 1)
- mix
- rinse with water (10 min), mix and dry

advantages:

- the acid can be used again
- macerated seeds can be stored for up to 1 month

disadvantages:

- the duration needs to be predetermined (according to the species and seed unit 10-60 min)
- sowing troubles (wet seeds)
- health safety and preservation

soaking in hot water (scalding)

 softening of seed coats by immersion in water at 4–5 times the volume of water 75-100 °C warm and gradual cooling (even repeatedly)

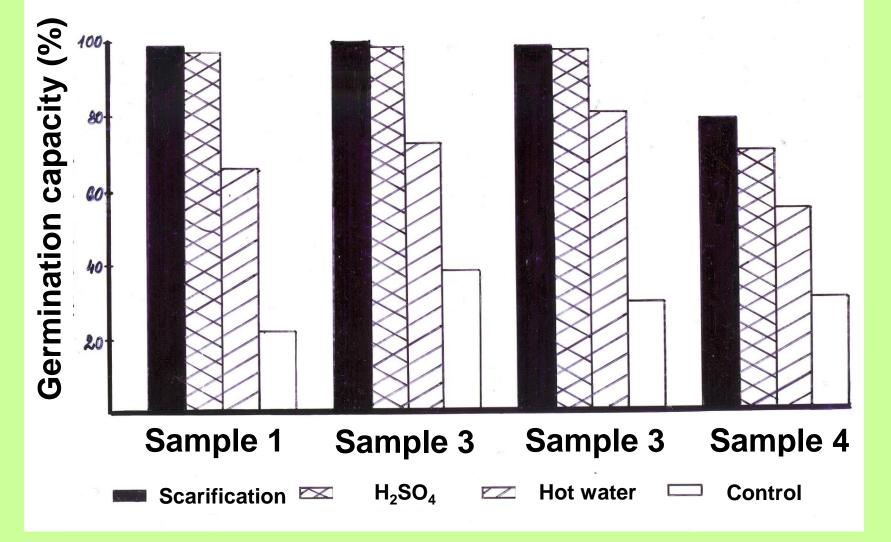
advantages:

- no equipment required
- easy, cheap

disadvantages:

- difficult standardization
- sowing troubles (wet seeds)

Germination capacity of four *Robinia pseudoacacia* samples after different pre-sowing treatment



How to determine a sufficient treatment duration?

samples of seeds treated at different times (scarification, maceration, hot water...) immersion in water for 1-5 days

the seed accepts water (bubbling) = sufficient treatment

- soaking in organic solvents
- alcohol, ether, xylene
- little practical significance

1.2 overcoming dormancy caused by the presence of growth inhibitors and/or the morphological state of the embryo

Objective: overcome the morphological and physiological barriers inside the seed (e.g. remove the inhibitors, allow the embryo to grow)

Stratification = exposing hydrated seeds to a specific temperature for a specific time



- older method
- mixing the seeds with the medium (ratio 1:1 to 1:3)
- humidity of the medium 60%

examples of media:

- highland peat
- sand
- perlite, vermiculite
- mixtures (sand : peat = 1 : 2)

function of the medium:

- maintains humidity
- provides air exchange
- removes heat
- isolates the seeds (infections)



- the mixture is stored in: - stratification containers



- stratification tanks

disadvantages:

- space requirements
- seeds germinate and emerge unevenly (non-uniformly)

stratification without a medium (pre-chilling)

- pre-hydrate the seeds
- insert into a PE bag or a crate

advantages:

- easy control and maintenance of humidity
- can prevent premature germination
- uniform germination and emergence

Conditions for stratification success

- sufficient water content in seeds
 - necessary for stratification with and without a medium

soaking → water : seeds = 3 : 1, duration 24–48 hours, temperature max. 15 °C higher temperature → fast water intake = danger of mechanical damage to the seeds

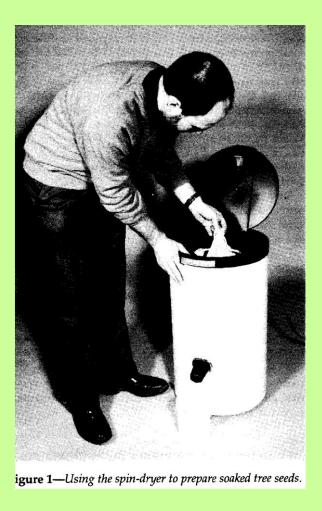
sprinkling \rightarrow **gentler** (suitable for long term storage of seeds)

"frozen" seeds need to be pre-acclimated for 24 hours at 5°C then soaked for 48 hours at max. 10 °C

- remove surface water before stratification without a medium

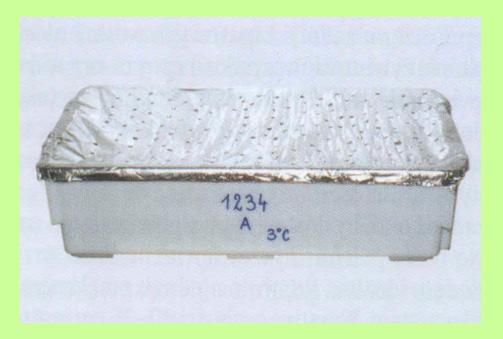
- a) surface drying
- b) in a nylon mesh in a centrifuge







- perforated lids
- tossing, opening of containers and hourglassing



temperature conditions for stratification – according to the cause of dormancy

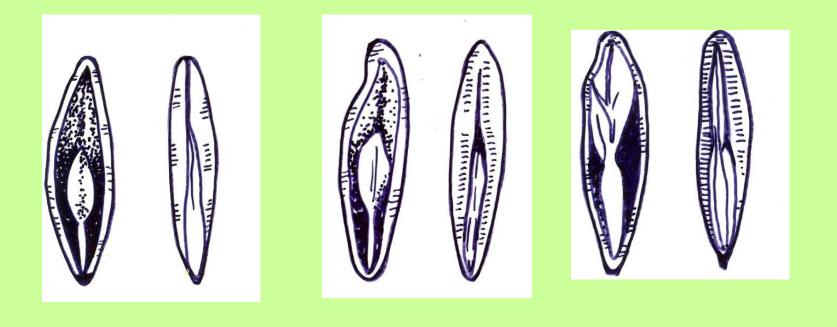
Cold stratification

- temperature 3–5 °C air-conditioned chamber or storage, or stratification pits in nature)
- cancels the effect of inhibitors

Warm-cold stratification

- warm phase (20–30 °C) the embryo grows
- cold phase (3–5 °C) cancels the effect of inhibitors
 - stratification length according to tree species

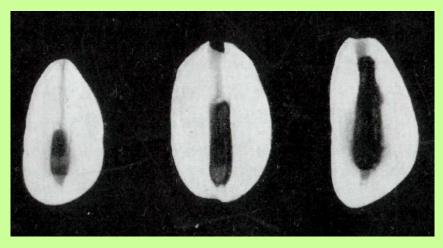
Embryo growing of Fraxinus - warm phase of stratification



Change of embryo size during the warm stage of stratification



llex - holly

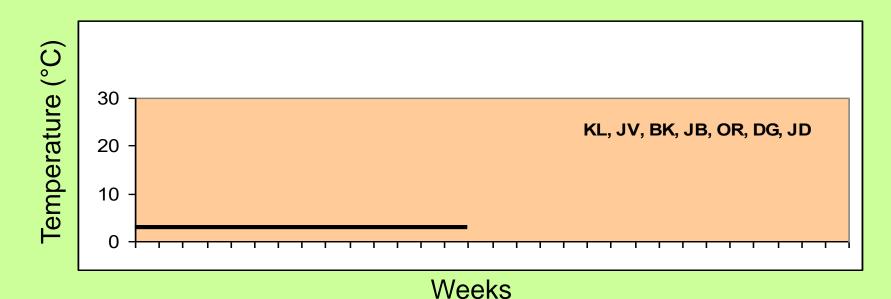


P. cembra – alpine pine

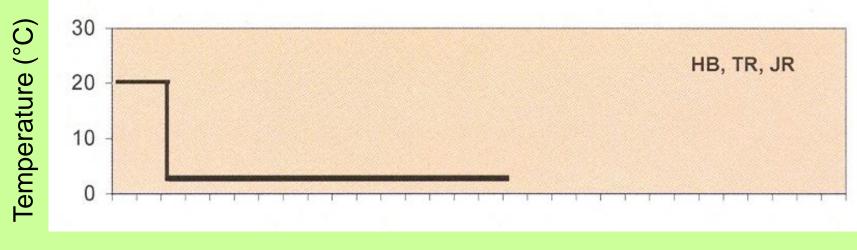
Stratification models

- cold only

fir, grand fir, pine (*Pinus strobus*), **Douglas fir**, maple (*Acer plataniodes*), maple (*Acer pseudoplatanus*), **beech**, walnut (*Juglans*), horsechestnut (*Aesculus*), pear (*Pyrus*), apple (*Malus*), chestnut (*Castanea*)



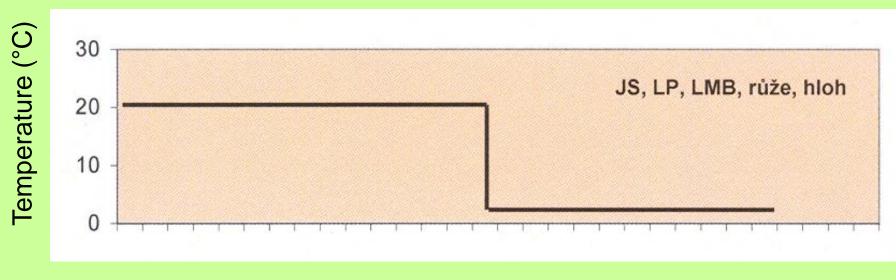
Warm-cold (short warm phase of 2–4 to 6 weeks, then cold phase)
hornbeam (*Carpinus betulus*), European mountain ash (*Sorbus sp.*), elder (*Sambucus*), lime (*Tilia cordata*)



Weeks

warm-cold (long warm phase of 2–6 months, then cold phase)

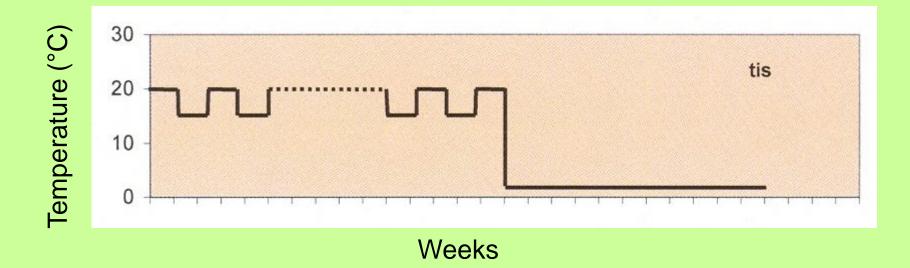
ash, lime (*Tilia plathyphylos*), alpine pine (Pinus cembra), rose, hawthorn (*Crataegus*), viburnum, eonymus, juniper



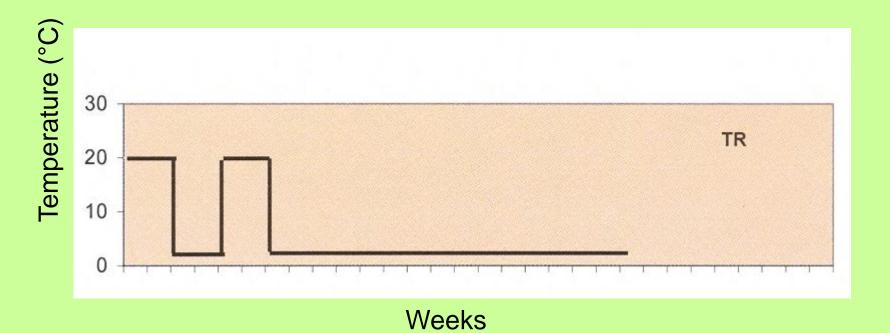
Weeks

 warm-cold (long warm phase with cyclic changes of temperatures 15/20 °C)

warm 6 months, temperatures change from 15° to 20 °C after 24 hours (yew (Taxus), dogwood (Cornus))



warm-cold (cyclic changing between warm and cold stages) cherry (Prunus avium)

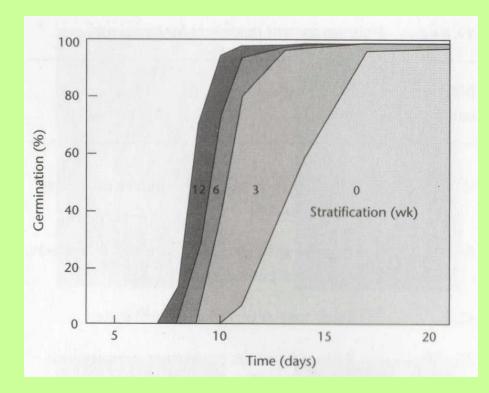


regular checks of the seeds and medium

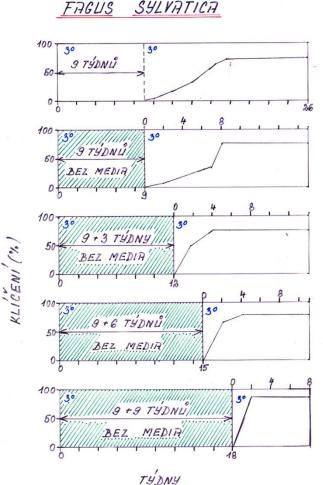
- necessary for stratification with and without a medium
- inspection of humidity, health condition
- aeration

Sufficiently long duration of stratification

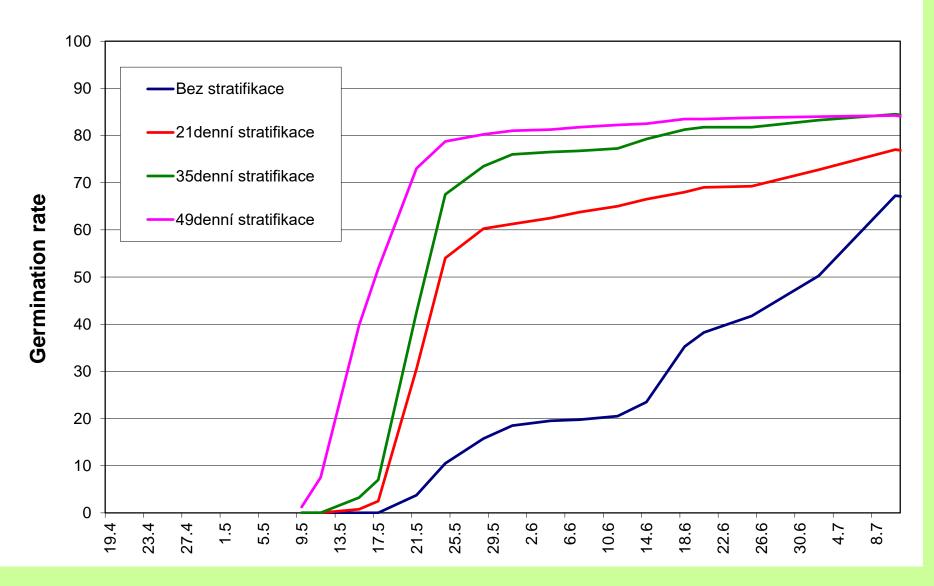
- depending on species, provenance, tree...
- the longer, the higher the yield of the seed and emergence rate

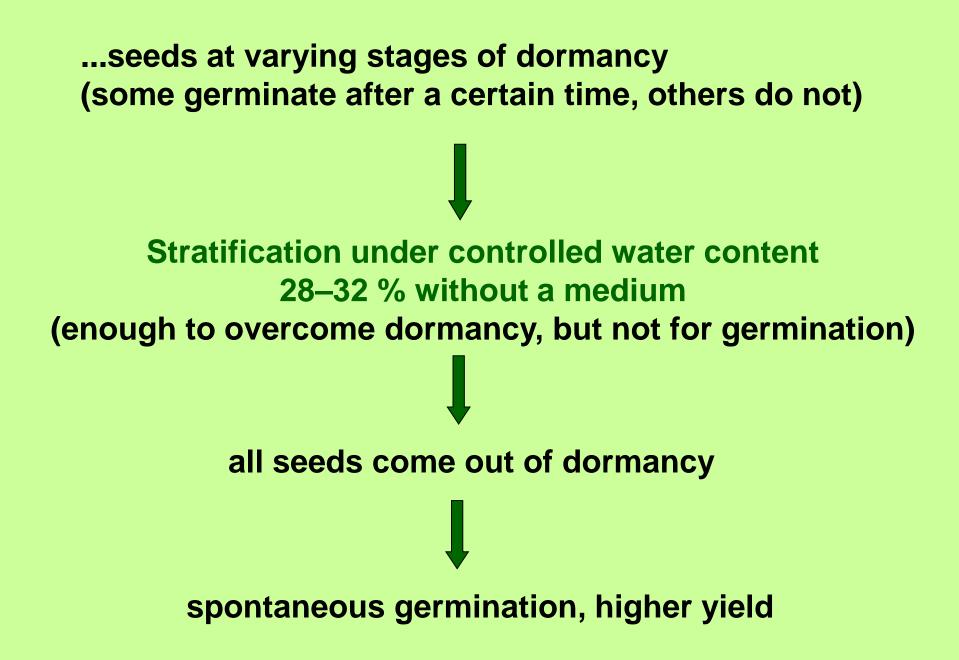


A. amabilis



Course of the emergence of Douglas fir seeds with a different pre-sowing treatment (April sowing)





How to reach the required water content?

The target humidity method

- only enough water is added to the seed to increase the humidity to the desired value
- calculation for the amount of water needed for hydration

$$H_2 = H_1 \frac{100 - V_1}{100 - V_2}$$

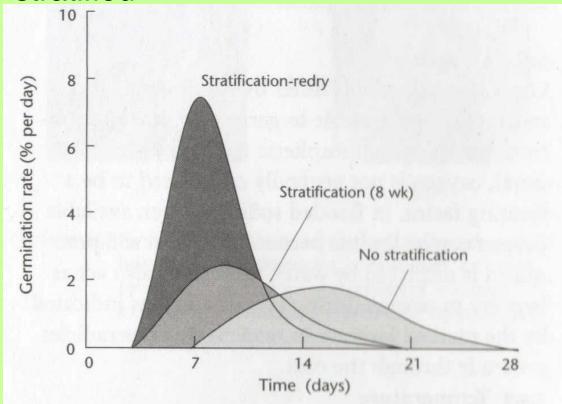
 H_1 original weight of the seeds H_2 weight of the seeds after water content adjustment V_1 original content of water in the seeds V_2 required content of water in the seeds Example: We have 500 kg of beechnuts with a water content of 8% and we need to increase the water content to 30%

H₁ = 500 kg V₁ = 8 % V₂ = 30 % $H_2 = 500 \frac{100 - 8}{100 - 30} = 657$

657 - 500 = 157 kg of water should be added to the beechnuts.

The stratification redry method

- the seeds are saturated with water and stratified for some time, then they are dried for a lower water content and further stratified



The effect of different stratification methods on germination rate *A. amabilis* (Leadem 1986) **Time of stratification**

- before sowing
- before storage
- during storage

Control stratification

 to determine the stratification duration and plan the sowing time

Procedure:

4 x 100 seeds into pots with a medium

x = days until germination 10% of the seeds (germ min. 5 mm)

required stratification duration: with a medium = x days without a medium = x + 2 (4) weeks Temperature conditions for the germination of stratified seeds

lime, hornbeam – can be sown in warm soil (20 °C)

beech – necessary to sow in cold soil (danger of secondary dormancy induction) or increase sowing depth (thermal insulation)

Framing lenght of stratification of forest tree seeds - conifers

Tree species	Stratification				
	warm		cold		
	(°C)	(weeks)	(°C)	(weeks)	
Pinus strobus			3 - 5	3 - 4	
Pseudotsuga menziesii			3 - 5	4	
Abies alba			3 - 5	3 - 4	
Abies grandis			3 - 5	3 - 4	
Tsuga canadensis			3 - 5	4	
Juniperus communis	20 - 30	8 - 13	3 - 5	13	
Taxus baccata	15 - 20	24	3 - 5	12	

Framing length of stratification of forest tree seeds - broadleaves

	Stratification				
Tree species	warm		cold		
	(°C)	(weeks)	(°C)	(weeks)	
Fagus sylvatica			3 - 5	6 - 12	
Acer pseudoplatanus			3 - 5	14	
Acer platanoides			3 - 5	12 - 18	
Acer campestre			3 - 5	12 - 24	
Sorbus aucuparia)			3 - 5	16 - 26	
Prunus avium			3 - 5	52	
Carpinus betulus	20	4	3 - 5	14 - 16	
Tilia cordata	20	16 - 18	3 - 5	14 - 18	
Tilia platyphyllos	15 - 20	16 - 18	3 - 5	20 - 24	
Fraxinus excelsior	15 - 20	6 - 16	3 - 5	16	

1.2.2 Soaking in oxygenated water

 replaces cold stratification (water at 5 °C saturated with O₂)

faster overcoming of dormancy (germinated seeds for "liquid" sowing)

1.2.3 Exogenous application of growth stimulators

gibberellins (GA3) and cytokinins (BAPs) can replace cold stratification (beech) or shorten the warm phase of stratification (ash)

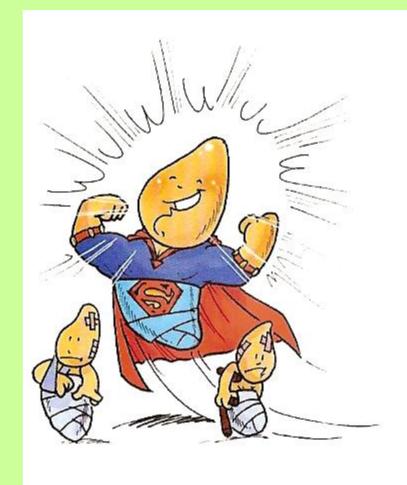
concentration 100-500 ppm

more important for research

2. Elimination of nonproductive seeds

nonproductive seeds:

- empty
- mechanically damaged
- full but lifeless (dead)
- damaged by insects



2.1 Removing empty seeds

a) pneumatically (causes losses)





- 2 principles:
- absorption principle
- density principle



2.1.1 Flotation on the absorption principle

 – for species with a small difference between the weight of full and empty seeds (spruce, pine, larch, pinus nigra)

- by absorbing water, the difference in weight increases
- full seeds settle (go down) and can be separated
- absorption time differs by species

The best fullness is reached at:

spruce – fractions sediment up to 8 hours from the start of flotation

pine, black pine – fractions sediment between 8-24 hours from the start of flotation

larch – fractions sediment after 20 min to 24 hours

- the seeds can be stored after drying

2.1.2 Flotation on the density principle

 the method uses solutions with a specific weight between the specific weight of full and empty seeds

- organic solvents (alcohol, hexane, tetrachloride, diethyl ether) or their mixtures

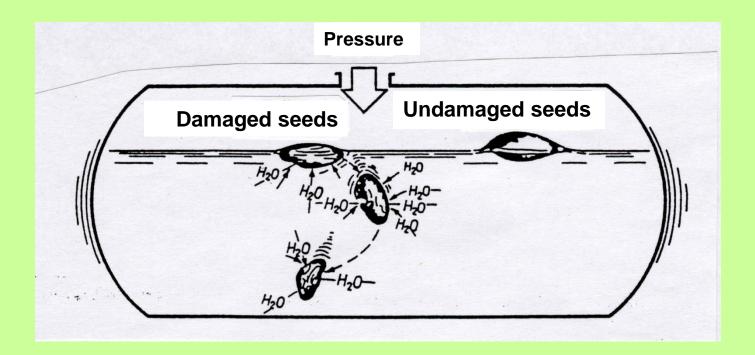
Flotation liquid	Density g/cm ⁻³	Species	Author
absolute alcohol	0.791	P. sylvestris P. abies	Schmidt 1929
diethyl ether	0.714	P. banksiana	Brown 1967
petrol ether	0.657	Abies, Cedrus, P. strobus P. sylvestris	Lebrun 1967
n-pentan	0.626	A.saccharum	Clayton 1969
chloroform	1.590 - 0.660	B. verrucosa	Björkroth 1972

 solution concentration and length of flotation are specific for the particular species: spruce, pine - abs. alcohol, max. 1 hour larch - 90% - abs. alcohol, max. 5 min

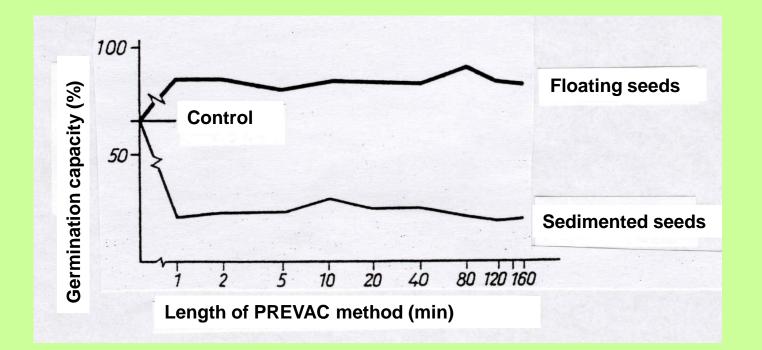
- flotation does not affect the storage life of seeds

 importance = removal of empty seeds and reduction of seed volumes for storage 2.2 Removing mechanically damaged seeds The PREVAC method (pressure or vacuum)

 mechanically damaged seeds immersed (put) in water and subjected to pressure or vacuum, they receive water faster than undamaged seeds



 after returning to normal atmospheric pressure, they sediment faster than undamaged seeds and can be separated



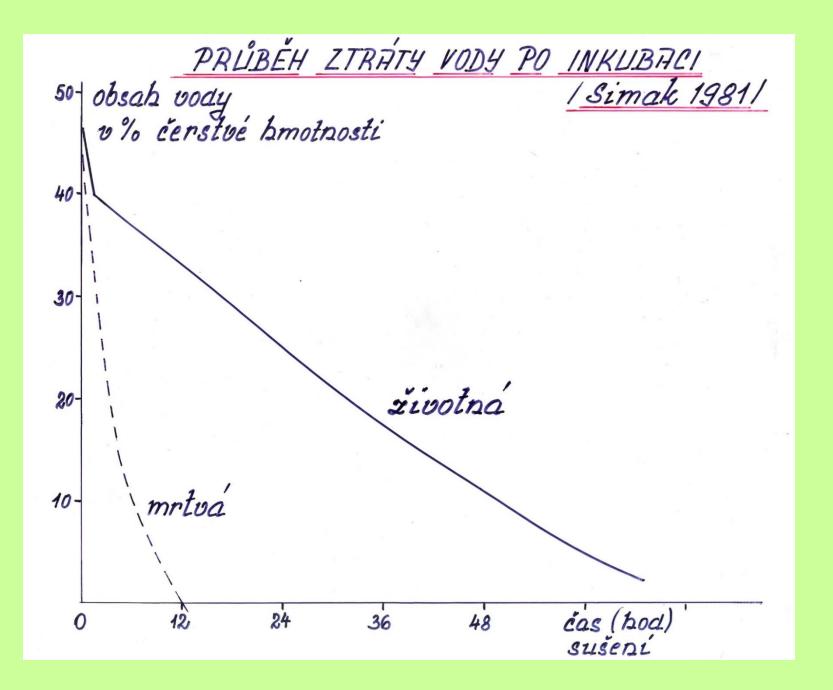
...a method suitable for spruce, pine and larch ...may also be used to remove empty seeds

Seed separation equipment using the Prevac method



2.3 Removing full lifeless (dead) seeds = "IDS method"

- the seeds receive water in a humid environment
- during drying, live seeds lose water more slowly than full lifeless (dead) seeds
- the different rates of water loss change the specific weight and allow separation



Procedure:

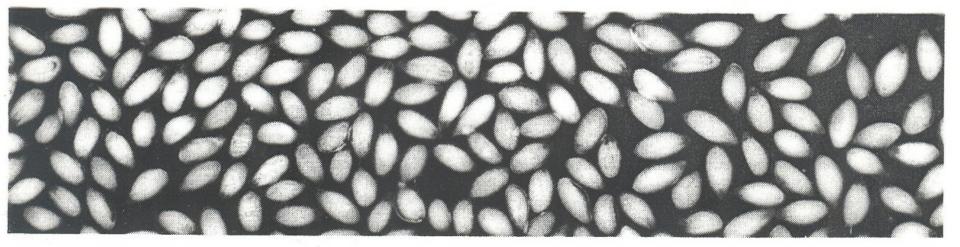
- I incubation
- RH 100%
- 3 days, temperature 15 °C
- increasing water content to approx. 35%
- **D** drying
- temperature 20-25 °C
- **S** separation
- flotation in water
- separation efficiency control by X-ray

Before IDS

RE!

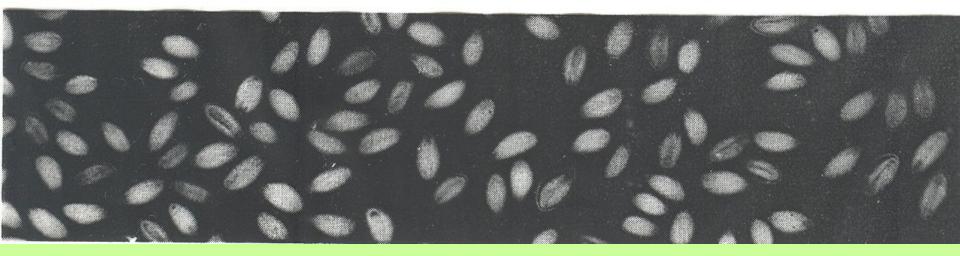


Incubation

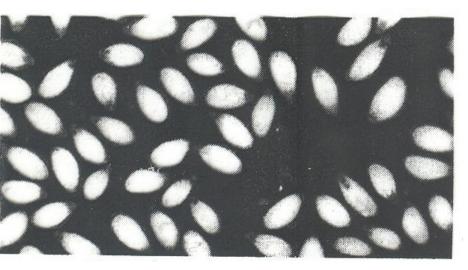


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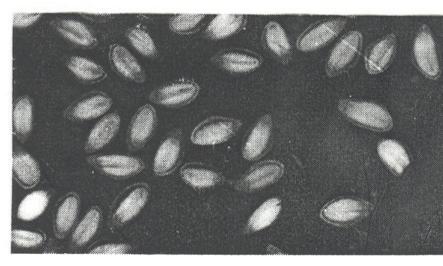
Drying



Separation



Live seeds



Dead seeds

Advantages of the IDS method

– reduces seed volumes for storage

improves the quality of seeds (a method suitable for spruce, pine and larch)

- seeds are suitable for precise sowing (into containers)
- allows better use of genetically valuable seeds
- modification of the IDS method for the separation of seeds damaged by insects (Douglas-fir)

3. Improving seed vitality

Seed vigour

 a set of properties that determine the ability of seeds to grow quickly and evenly and develop into seedlings over a wide range of environmental conditions



What determines seed vigour?



- **1. genotype** (self-pollination)
- 2. conditions during seed development and maturation (length of the veg. period, temperature, precipitation, nutrition of the parent tree...)
- 3. human activity

(time of collection – maturity, conditions of seed processing, storage...)

Seed vigour = a result of the interaction of genetic constitution and conditions during seed development and processing Methods to increase seed vigour

3.1 Treatment with chemicals

anorganic substances

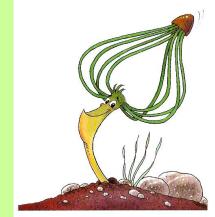
- KMnO₄, KNO₃, ZnSO₄, KBr, boric acid...

organic substances

- citric acid, tartaric acid, diacetobutane

growth substances (non-dormant seeds!)

- all types are tested
- GA usually the most effective
- concentration 10-500 mg/l



3.2 Treatment with different types of energy

- Thermal shocks
 - from the 22nd to the 28th day of beechnut stratification, increase the temperature from 3 °C to 15 °C for 24 hours every fifth day

- synchronization of germination and 10% yield increase
- ultrasound
- He-Ne laser
- ionizing radiation

The following applies to both methods of stimulation 3.1 and 3.2:

- seed units react differently
- an increase of germination energy, germination capacity, emergence rate can be reached
- difficult comparability and reproducibility
- the concentration or intensity and length of action must be tested for each seed lot
- the stimulation effect cannot be generalized
- only of research importance

3.3 Physiological treatment

the objective:

 speed up water intake by seeds before sowing and shorten the interval from sowing to emergence

suitable for stored seeds

homogenous sowing

3.3.1 Soaking in water

- water 17-20 °C
- drying = thin layer, max. 20 °C, RH 45-65%
- sow immediately

spruce - optimum length of soaking 24 hours drying 12-24 hours

larch - optimum length of soaking 24 hours drying 24 hours

pine - positive effect only on germination energy - not recommended The effect of **spruce** seed drying time after 24 or 48 hours of soaking in water on germination energy and germination capacity (Hrabí 1990)

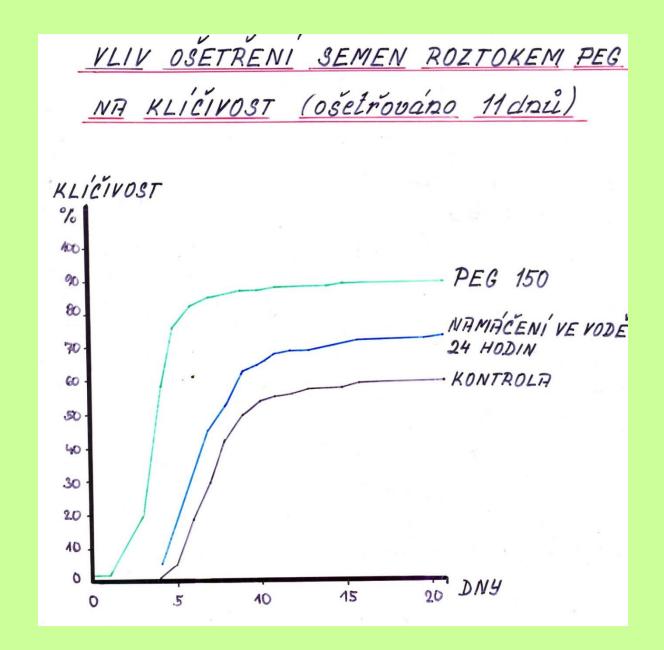
Drying	Soaking	24 hours	Soaking 48 hours		
length	Germination Energy (%)	Germination capacity (%)	Germination energy (%)	Germination capacity (%)	
Control	0	60	0	60	
0 hours	12	44	27	69	
6 hours	33	75	34	72	
12 hours	20	79	16	63	
24 hours	18	79	15	58	
36 hours	14	75	5	53	
48 hours	16	66	4	52	

Germination rate of spruce seeds (in %) after soaking (Hrabí 1990)

Seed	Variant	Date			
unit		11/05	18/05	25/05	01/06
Samo	Control	17	41	43	44
Spruce 1	24 hours of soaking 12 hours of drying	37	56	55	56
	Control	1	17	25	26
	24 hours of soaking 12 hours of drying	9	41	44	43

3.3.2 Osmotic stimulation

- seeds bubble in aqueous osmotic solutions (PEG, Carbowax)
- after receiving water, the initial stages of germination take place in the seed, but the radicle is inhibited during growth
- immediate germination and better germination under lower temperatures after the removal of osmotics
- the concentration of osmotics for the particular species and provenance must be predetermined
- good results in spruce, pine, larch, Douglas-fir, black pine



3.3.3 Pre-chilling stimulation (stratification of non-dormant seeds)

- -- soak in water at 3-5 °C for 24 hours, pour water
- store at 3-5 °C for 3-4 weeks
- surface dry and sow

Importance:

reduces the sensitivity of germinating seeds

to temperature

- increases germination rate
- ensures uniform emergence

4. The elimination of surface and internal mycoflora

4.1 Surface sterilization

- = removing fungal spores from the seed surface
- 4.1.1 Rinsing with running water (24 hours)
- 4.1.2 Soaking in oxidizing agents
 - * sodium hypochlorite NaClO (5 %)
 - = bleach for clothes washing
 - soak the seeds for 10 min, stratification or sowing after rinsing
 - suitable for seeds with strong seedcoat (pine, Douglas-fir), not suitable for fir, spruce, larch

The germination capacity of coniferous seeds surfacesterilized with a mixture of 2 parts bleach and 3 parts water

Tree species	Germination (%)		
	Check	Bleach	
Pinus nigra	62	69	
Pseudotsuga menziesii	73	82	
Pinus ponderosa	63	81	
Pinus sylvestris	59	87	

hydrogen peroxide Douglas-fir, spruce 30% H₂O₂, 30 min Douglas-fir 3% H₂O₂, 4 hours fir 1% H₂O₂, 1 hour



- 4.2. Pickling (seed dressing)
- surface treatment of seeds
- the objective is to prevent the germination of spores and conidia
- less is enough to control the vegetative mycelium

4.2.1 Pickling with fungicidal agents

- a) before sowing
- **b) before stratification**
- c) before storage

Pickling methods

Dry

- application of powder pickling agents
- 0.5-0.7%, max. 1 % of seed weight
- pickling barrels

Wet

- application of wet pickling agents

The slurry method

- suspension application
- wettable pickling agent : water = 1 : 2
- adhesive agents can be added

Pickling agents: List of authorized plant protection products (State Phytosanitary Administration)

4.2.2 "Pickling" with a biologic preparation

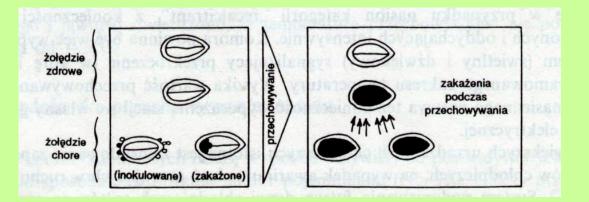
use of mycoparasitic fungi (parasitic on other fungi)
Trichoderma harzianum (Supresivit)
Phytium oligandrum (Polyversum)
Bacilus subtilis (Ibefungin)

- usually 2 g per 1 kg of seeds
- increases seed yield

4.3 Thermotherapy

- physical method of seed protection
- control not only superficial, but also internal pathogenic mycoflora
- utilizes the different sensitivity of the seeds and fungi to heat
- used for beech (x Rhizoctonia solani) oak (x Ciboria batschiana)

opadanie żołędzi rozsiewanie zarodników rozsiewanie zarodników









Heat can be applied in the form of

- dry air
- steam
- soaking in warm water (best)

The technical conditions of thermotherapy

- fast reaching target temperature
- movement (circulation) of water
- maintenance of a constant temperature

The advantages of thermotherapy

- no residues left
- treats even deep infections
- partially damaged sections are recovering

The results of *Quercus petraea* acorn thermotherapy (Delfs-Siemers 1993)

Variant	Germination capacity after collection (%)	Germination capacity after 6 months storage (%)
Control	71	4
41°C, 2 hours	60	57

Note: The values are affected by 30% of germinated acorns before thermotherapy

Thermotherapy for acorns

- before storage
- 2 hours in warm water at 41 °C
- do not treat germinated seeds
- sessile oak more sensitive than pedunculate oak

Thermotherapy for beech nuts

- before stratification or storage
- 1 hour in water at 41 °C





Acorn and beechnut thermotherapy line in State seed company Týniště nad Orlicí





Flotation tank

Thermotherapy boiler





Apparatus for the surface drying of acorns

5. Improving the sowing properties of seeds

5.1. Pelletizing (seed coating)

– the objective is to increase the size of small seeds and/or to make their shape more regular

Pelletized seeds

= spherical units containing one seeds, the shape of which is not clear or remains unchanged



Pelletized beech seeds



Pelletized spruce seeds

Requirements for coating materials

- adherence to the seed
- appropriate response to moisture changes
- must not prevent the penetration of O_2 or the growth of the radicle
- must not negatively affect the seeds during germination or storage

The composition of coating materials

basic substance (methylcellulose, kaolin, wood flour, sand, peat...)

binders (organic substances, starch)

active ingredients (growth substances, pesticides, fungicides, nutrients...)

- the composition is usually patent protected
- in forestry, pelletizing is used only seldom

5.2. Sorting of seeds by absolute weight

- there is a correlation between seed size and seedling initial growth
- the effect is different in species with small and large seeds

species with large seeds (oak, chestnut...)

- differences in height are seen up to 8 years

The average heights of oak seedlings grown from small, medium and large acorns up to the age of 8 (Eitingen ex Rohmeder 1972)

	Seedling height (cm)		Seedling height (%)			
Age	Small 2.0 g	Medium 5.0 g	Large 8.0 g	Small 2.0 g	Medium 5.0 g	Large 8.0 g
1+0	8.5	11.6	15.5	55	75	100
3+0	22.4	33.0	41.0	55	80	100
5+0	44.1	68.4	82.5	53	83	100
8+0	109.1	136.7	143.5	76	95	100

species with small seeds (spruce, pine, Douglas-fir...)

- differences in height disappear at age 2 to 3

Absolute weight	Spruce seedling height in cm (Muhle et al. 1985)		
(g)	Year 1	Year 2	Year 3
10.8	4.1	7.00	13.90
9.2	4.0	7.57	14.47
7.0	3.9	8.08	13.00
5.7	3.6	7.57	12.73

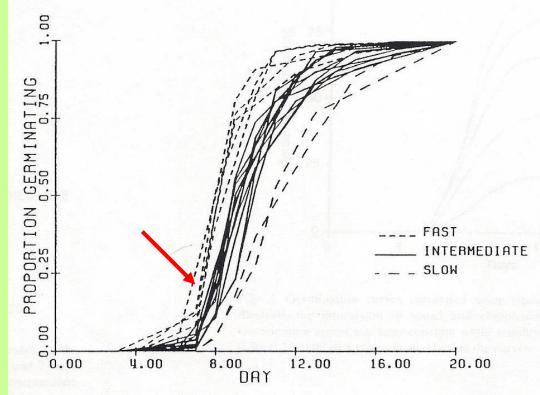
Absolute weight	Pine seedling height in cm (Muhle et al. 1985)		
(g)	Year 1	Year 2	Year 3
11.4	4.6	10.2	21.0
9.1	4.7	10.6	19.7
6.6	4.0	10.0	20.35

 uniform seedlings can be obtained by the separated sowing of the weight fractions of the seed

- this reduces losses caused by competitive relations

5.3 Sowing clone families

- use for seeds from seed orchards
- collection of seeds from individual clones (lower genetic variability)



Seed germination of 19 Douglas-fir families

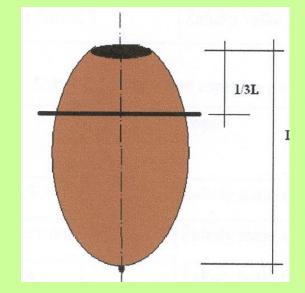
half-sister progeny have a similar growth rhythm

 higher uniformity of seedlings and lower losses due to competitive relations

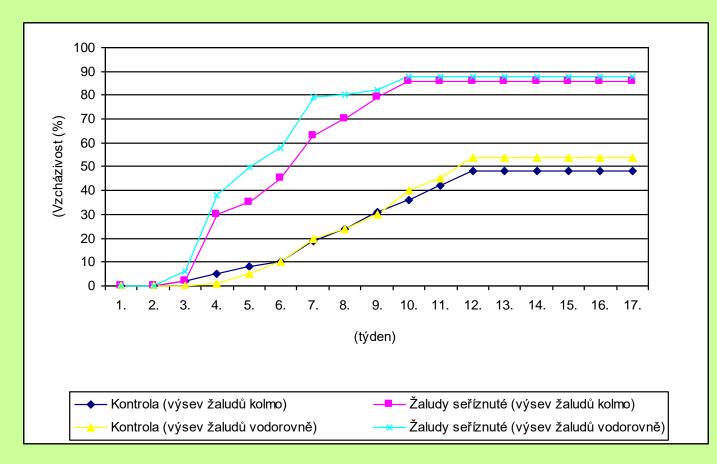
5.4 Cutting of acorns

use in the cultivation of contained seedlings

 cutting 1/3 of the acorn from the base, sowing horizontally, tip in the middle of cell in container



faster emergence (by 2–3 weeks) than intact acorns



- no height difference in seedlings
- higher efficiency of use of containers

Thanks for your attention

