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Conditions for dormancy breaking and germination of European hophornbeam (*Ostrya carpinifolia* Scop.) seeds. Part II. Seeds stored for 12 months

Abstract

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The germinative capacity of European hophornbeam seeds increases after one-year storage at −3°C compared to seeds stored at −3°C for a month. This concerns seeds subjected to a warm-followed-by-cold stratification with the warm phase at 20°C (2–6 weeks) and the cold phase at 3° or 5°C. Each duration of the warm phase, i.e. 2, 4 and 6 weeks was equally successful and the temperature of the cold phase was as effective at 3° as at 5°C. The most successful germination temperatures were an alternating 3°~20°C one and constant 20°C. The germinative capacity of full seeds selected out after collection by floation first in water and then in ethanol stored for a year at −3°C and stratified was significantly lower than for seeds floated only in water. For half full seeds a temperature of 3°C proved more favourable for one-year storage than −3°C.

Additional key words: stratification, floatation.

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INTRODUCTION

The present work is a continuation of last years studies conducted shortly after seed collection (Chmielarz 1990). In the present experiments use was made of seeds stored under controlled conditions for 12 months expanding the scope of thermal conditions used for stratification.

There is no information in the literature about the possibility of storing *Ostrya carpinifolia* seeds. *Ostrya virginiana* has been succesfully stored over one winter (Schopmeyer and Lask 1974). A related genus, *Carpinus betulus*, has

seeds that can be stored partially dried in sealed containers in cool conditions for 2–3 years (Holmes and Buszewicz 1958).

MATERIALS AND METHODS

THE ORIGIN OF SEEDS

Seeds of the European hophornbeam used in the study originate from central Italy, from a place called Spello near Assisi (190 m elev.). Nutlets collected in the fall of 1988 have been obtained on January 11th 1989 from the Societá Agricola e Forestale per le Plante di Cellulosa e da Carta, located in Rome.

SEED HANDLING BEFORE STRATIFICATION

The nutlets, henceforth referred to as seeds, have been stored 3 months after collection, still in Italy in uncontrolled conditions. They reached us in tightly closed polythene bags, in which we stored the seeds temporarily at −3°C for a month, after which they were separated by floatation, (first using water and then ethanol, or using water only), to give us full, half-full seeds and empty seeds (Chmielarz 1990). The full and half-full seeds were dried after floatation and stored separately in sealed bottles at 3°C and −3°C for 12 months. (To the storage for 12 months in Kórnik, one should always add the 3 months of storage in Italy in conditions unknown to us and the 1 month of provisional storage in Kórnik at −3°C. When speaking of non-stored seeds always the seed handling prior to the experiment has to be kept in mind).

INITIAL SEED QUALITY

Moisture content of seeds was determined directly before stratification by drying them in an oven at 105°C for 24 h (3 × 30 seeds). The results obtained refer to the fresh weight of whole nutlets. For the evaluation of seed quality the cutting test was used (4 × 50 seeds). The moisture content of seeds prior to stratification was 7.5–7.7% for full seeds, and 8.0–8.1% for half-full seeds both before and after storage.

EXPERIMENTAL DESIGN

Experiment no. I. Aim: To compare the germinability of full seeds stored for 12 month at a temperature of −3°C with the germinability of those that were not stored.

For experiment I use was made of full seeds separated out by floating in water. The seeds, stored for 12 months, have been subjected to a warm-followed-by-cold stratification with the warm phase at 20°C lasting 2, 4 and 6 weeks and cold phase at 3° and 5°C. Germination tests for the seeds

that were not stored were made independently for each stratification variant at a constant temperature of 3°C for 12 weeks, at 20°C for 10 weeks and in the alternating temperature of 3°~20°C (16+8 hr/cycle) for 10 weeks. Germination tests of seeds stored for 12 months were run at 3°C for 24 weeks at 20°C for 8 weeks, and at the alternating temperature 3°~20°C for 8 weeks (Fig. 1). In Fig. 1, 2 and 3 the storage of seeds before the experiments in uncontrolled conditions for 3 months and provisionally at −3°C for a month is not indicated.

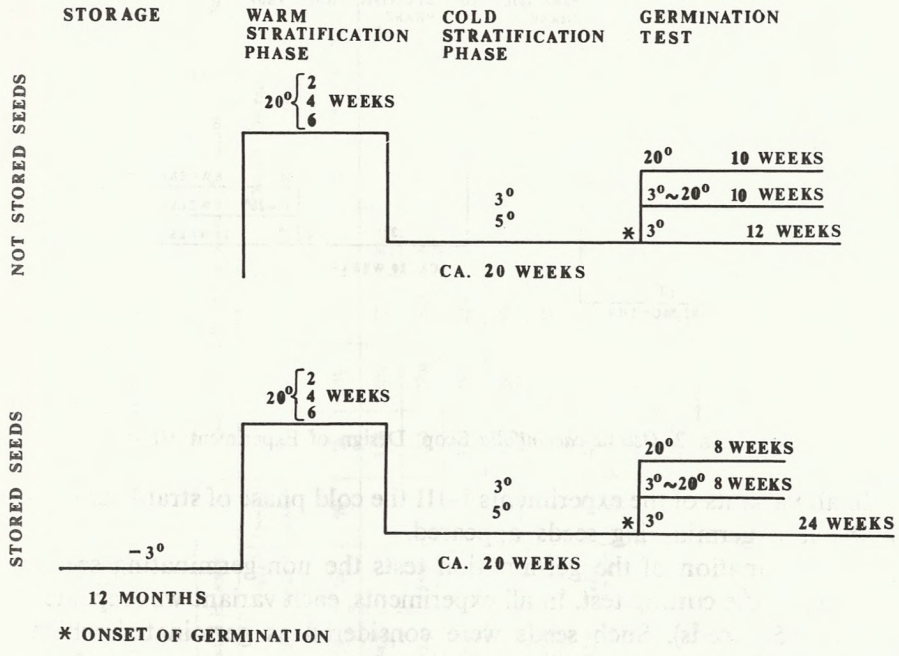

Fig. 1. *Ostrya carpinifolia* Scop. Design of Experiment I and II

Experiment II. Aim: To study the effect of ethanol used for the floatation of nutlets on the germinability of full seeds after storage for 12 months at a temperature of −3°C and warm-followed-by-cold stratification.

Seeds were compared which were full as indicated by floatation in water and those floatated both in water and in ethanol. A warm-followed-by-cold stratification was applied with the warm phase at 20°C lasting 2, 4 and 6 weeks and the cold phase at 3° and 5°C for about 20 weeks. Germination tests after each stratification were conducted independently from each other at 3°C for 24 weeks and at 20°C for 8 weeks as well as at an alternating temperature of 3°~20°C (16+8 hr/cyle) for 8 weeks. (Fig. 1, lower part 1).

Experiment III. Aim: Comparison of germinability of half-full seeds stored for 12 months at 3° and −3°C.

For experiment III use was made of half-full seeds obtained by floatation c first in water and then in ethanol. Such seeds were used because of the lack of a sufficient number of full seeds. Stratification was performed in three thermal arrangements 15°/3°, 20°/3°, 25°/3°C with the warm phase lasting 4 weeks and the cold one ca. 20 weeks. Germinations tests were run as in Exp. II, (Fig. 2).

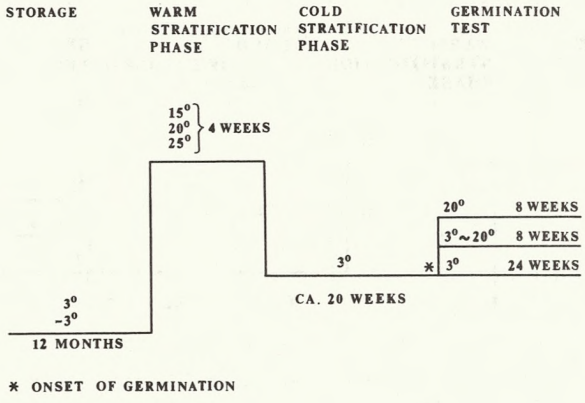

Fig. 2. *Ostrya carpinifolia* Scop. Design of Experiment III

In all variants of the experiments I–III the cold phase of stratification lasted till the first germinating seeds appeared.

On termination of the germination tests the non-germinating seeds were subjected to the cutting test. In all experiments, each variant was repeated four times (4 × 50 seeds). Such seeds were considered as germinated which had a radicle at least 3 mm long.

In the experiments discussed the germination test was the final result. No attempts were made to study seedling emergence, neither in the laboratory nor in the nursery.

Results of the experiments transformed from percentages to arcus sinus have been subjected to variance analysis and the significance of differences between mean values have been checked with a Duncan test at an $\alpha = 0.05$ level of significance.

RESULTS

Exp. I. Germinative capacity of seeds stored at −3°C for 12 months was significantly higher compared to seeds that were not stored in such conditions

Table 1

Ostrya carpinifolia Scop. Germination capacity of full seeds floatated in water only or in both water and ethanol and then stored 12 months at −3°C, compared to seeds that were not stored (from Chmielarz 1990). The seeds were subjected to a warm-followed-by-cold stratification with the warm phase at 20°C lasting 2, 4 or 6 weeks and the cold phase at 3° or 5°C. The germination tests were conducted at an alternating temperature of 3°∼20°C and at constant temperatures of 3°C and 20°C, starting from the moment when first seeds started germinating in the cold phase of stratification

Stratification			Seeds not stored floated in water						Seeds stored floated in water and ethanol						Seeds stored floated in water					
Temp. of warm phase %	Temp. of cold phase %	Duration of warm phase weeks	3° %	3°∼20° %	20° %	$\bar{x}$ %	Overall mean 1 %	Overall mean 2 %	3° %	3°∼20° %	20° %	$\bar{x}$ %	Overall mean 1 %	Overall mean 2 %	3° %	3°∼20° %	20° %	$\bar{x}$ %	Overall mean 1 %	Overall mean 2 %
		2	33.0	36.0	35.5	34.7			31.5	30.5	32.5	31.5			25.5	39.3	34.3	33.0		
	3°	4	33.0	31.0	36.5	33.8	34.4		26.5	28.0	30.5	28.2	29.7		32.0	31.5	36.5	33.3	33.0	
		6	32.0	38.0	34.5	34.8			31.0	28.0	29.0	29.3			30.0	35.5	33.0	32.8		
20°		$\bar{x}$	32.6	35.0	35.5			30.5	29.7	28.8	30.7			29.7	29.2	35.4	34.6			33.9
		2	17.0	35.5	18.5	23.6			29.5	29.0	32.5	30.2			29.5	36.0	35.5	33.7		
	5°	4	30.5	41.5	26.5	32.8	26.7		32.0	30.5	28.0	30.2	29.7		31.0	38.5	30.5	33.3	34.9	
		6	14.0	35.0	22.5	23.8			32.0	29.5	24.5	28.7			34.0	40.5	38.5	37.7		
		$\bar{x}$	20.5	37.3	22.5				31.2	29.7	28.3				31.5	38.3	34.8			
Overall mean 1			20.5	37.3	22.5				30.5	29.3	29.5				30.4	36.9	34.7			

Experiment I

Experiment II

(Table 1). Variance analysis was prformed basing on all values of germinability. (Table 2).

Table 2

Ostrya carpinifolia Scop. Variance analysis of germinative capacity of full seeds stored for 12 months at −3°C, compared to seeds that were not stored (from Chmielarz 1990). The seeds were subjected to a warm-followed-by-cold stratification with the warm phase at 20°C lasting 2, 4 or 6 weeks and the cold phase at 3° or 5°C. The germination tests were conducted at an alternating temperature of 3°~20°C and at constant temperatures of 3°C and 20°C, starting from the moment when first seeds started germinating in the cold phase of stratification

Source of variation	Degrees of freedom	Sum of square	Mean square	F
Storage (A)	1	190.12	190.12	9.91**
Temp. of cold stratification phase (B)	1	155.05	155.05	8.08**
Duration of warm phase (C)	2	42.55	21.27	$1.11^{N.S}$
Tmep. of germination (D)	2	655.89	327.95	17.10**
A × B	1	364.74	364.74	19.02**
A × C	2	82.70	41.35	$2.16^{N.S}$
A × D	2	70.82	35.41	$1.85^{N.S}$
B × C	2	58.50	29.25	$1.53^{N.S}$
B × D	2	249.96	124.98	6.52**
C × D	4	82.91	20.73	$1.08^{N.S}$
A × B × C	2	154.79	77.40	4.04*
A × B × D	2	159.46	79.73	4.16*
A × C × D	4	44.41	11.10	$0.58^{N.S}$
B × C × D	4	80.37	20.09	$1.05^{N.S}$
A × B × C × D	4	41.40	10.35	$0.54^{N.S}$
ERR	108	2071.41	19.18	

** significant at = 0.01
* significant at = 0.05
N.S. not significant

Overall 5°C cold stratification was better than 3°C but this was true only for the non-stored seed as reported earlier (Chmielarz 1990), after storing the difference disappearing, whence the A × B interaction.

No significant differences in germination were observed regardless whether the warm phase of 20°C lasted 2, 4 or 6 weeks (33.4%, 33.3% and 35.2% respectively). Thus the differences observed before storage were not confirmed after storage.

Of the germination test temperatures the alternating one 3°~20°C proved better than the constant 20° and 3° ones (37.1%, 28.6%, 25.5% respectively) these differences being significant both with and without storage. After storage

the 3°~20°C temperature was better for germination than temp. of 3°C but not significantly different from temp. 20°C. Similarily the levels of germinative capacity were not significantly different for test temperatures 3° and 20°C.

Exp. II. Reduced germinability was observed of full seeds which before storage were treated with ethanol. (Tables 1 and 3). The statistical analyses

Table 3

Ostrya carpinifolia Scop. Variance analysis of germinative capacity of full seeds floatated in water only or in both water and ethanol and then stored 12 months at −3°C. The seeds were subjected to a warm-followed-by-cold stratification with the warm phase at 20°C lasting 2, 4 or 6 weeks and the cold phase at 3° or 5°C. The germination tests were conducted at an alternating temperature of 3°~20°C and at constant temperatures of 3° and 20°C, starting from the moment when first seeds started germinating in the cold phas of stratification

Source of variation	Degrees of freedom	Sum of square	Mean square	F
Floatation (A)	1	228.72	228.72	11.28**
Temp. of cold stratification phase (B)	1	6.79	6.79	$0.34^{N.S}$
Duration of warm phase (C)	2	10.40	5.20	$0.26^{N.S}$
Temp. of germination (D)	2	55.43	27.72	$1.37^{N.S}$
A×B	1	16.35	16.35	$0.81^{N.S}$
A×C	2	33.74	16.87	$0.81^{N.S}$
A×D	2	159.69	79.85	3.94*
B×C	2	10.69	5.05	$0.25^{N.S}$
B×D	2	26.16	13.08	$0.65^{N.S}$
C×D	4	50.68	12.67	$0.62^{N.S}$
A×B×C	2	37.85	18.93	$0.93^{N.S}$
A×B×D	2	0.48	0.24	$0.01^{N.S}$
A×C×D	4	43.31	10.83	$0.53^{N.S}$
B×C×D	4	54.47	13.62	$0.67^{N.S}$
A×B×C×D	4	49.22	12.30	$0.61^{N.S}$
ERR.	108	2189.69	20.27	

** significant at=0.01
* significant at=0.05
N.S. not significant

confirm significance of the differences observed on the overall mean. Also ethanol seems to have eliminated the effect of germination test temperatures.

Exp. III. After a year of storing half-full seeds at 3° and −3°C it was found that a temperature of 3°C was more favourable (Tables 4 and 5). The success of the three temperatures of the warm phase tried (15°, 20° and 25°C) depended

Table 4

Ostrya carpinifolia Scop. Germination capacity of half-full seeds stored for 12 months at a temperature of 3° and −3°C and then subjected to a warm-followed-by-cold stratification with the warm phase at 15°, 20° and 25°C and the cold phase at 3°C. The germination tests were performed at an alternating temperature of 3°∼20°C (16+8 hr./cycle) or at constant temperatures 3° and 20°C. Values within a column marked with the same letter do not differ significantly (Duncan test)

Stratification		Seeds stored at 3°C					Seeds stored at −3°C				
Temp. of warm phase duration for 4 weeks °C	Temp. of cold phase duration for 20 weeks °C	germinability at									
		3° %	3°∼20° %	20° %	$\bar{x}$ %	Overall mean %	3° %	3°∼20° %	20° %	$\bar{x}$ %	Overall mean %
15°	3°	21.0	19.0	23.0	21.0 a	19.1	17.5	18.5	14.5	16.8	16.3
20°		17.5	29.0	18.0	21.5 a		14.5	17.5	12.5	14.8 a	
25°		11.5	19.0	13.5	14.7 b		15.0	22.0	14.5	17.2 a	
	$\bar{x}$	16.7	22.3	18.2			15.7	19.3	18.7		

on the storage temperature. After one year of storage at 3°C the most effective temperatures of the warm phase were 15° and 20°C. Germinability was significantly lower when the temperature of the cold phase was 25°C. After one year of storage of seeds at −3°C all three temperatures of the warm phase of stratification 15°, 20°, 25°C gave low germinabilities. Most effective of the three germination test temperatures (3°, 3°∼20° and 20°C) for the half-full seeds was the cyclically alternating temperature of 3°∼20°C.

Table 5

Ostrya carpinifolia Scop. Variance analysis of germinative capacity of half-full seeds stored for 12 months at a temperature of 3° and −3°C and then subjected to a warm-followed-by-cold stratification with the warm phase at 15°, 20° and 25°C and the cold phase at 3°C. The germination tests were performed at an alternating temperature of 3°∼20°C (16+8 hr./cycle) or at constant temperatures 3° and 20°C. Values within a column marked with the same letter do not differ significantly (Duncan test)

Source of variation	Degrees of freedom	Sum of square	Mean square	F
Temperature of storage (A)	1	80.41	80.41	5.02*
Temp. of warm stratification phase (B)	2	70.95	35.47	$2.21^{N.S}$
Temp. of germination (C)	2	203.27	101.64	6.34**
A×B	2	158.85	79.42	4.96*
A×C	2	22.19	11.09	$0.69^{N.S}$
B×C	4	109.82	27.46	$1.71^{N.S}$
A×B×C	4	49.03	12.26	$0.77^{N.S}$
ERR	54	864.99	16.02	

** significant at = 0.01
* significant at = 0.05
N.S. not significant

DISCUSSION

In an earlier study (Chmielarz 1990) it was reported that dormancy of European hophornbeam (stored shortly between collection and setting up of the experiment) was successfully overcome by a warm-followed-by-cold stratification with a 4-week warm phase at 20°C or 20°~30°C (16+8 hr./cycle) and the following cold phase at 3°C lasting until the onset of germination i.e. for about 20 weeks. After a further one-year storage at −3°C the tested thermal regimes for stratification were expanded with 15° and 25°C in the warm phase for half-full seeds. It turned out that these two temperatures are just as successful for the warm phase as 20°C but only so when the storage was conducted at −3°C. When the storage temperature was 3°C the higher, 25°C temperature of the warm phase proved less statisfactory and the temperatures 15° and 20°C were more effective. After a year of storage of full seeds at −3°C the temperature of the cold phase 5°C proved as successful as 3°C. Of the germination test temperatures used (3°, 3°~20° and 20°C) for seeds stored only shortly after collection the one reported (Chmielarz 1990) as best was the alternating one 3°~20°C. In the present study (Table 1) when full seeds stored for one year at −3°C were used the constant temperature of 20°C proved as effective in the germination test as the alternating 3°~20°C temperature. At the same time germinative capacity of seeds held at 3° and 20°C did not differ significantly. The increased (compared to last year) germinability of seeds tested at constant temperatures of 3° and 20°C was most likely caused by the year-long storage of seeds at −3°C. In the case of the 3°C germination test temperature, this could possibly have been also caused by the extended duration of this phase to 24 weeks. In the case of half-full seeds the effectiveness of the germination phase temperatures was similar as in the full seeds. As can be judged from Fig. 3 the seeds germinated earliest in the constant 20°C temperature, already in the first week. In the alternating 3°~20°C germination temperature the germination was fastest in the first week but it was extended over the period of the first 4 weeks. At the constant 3°C the germination took longest. For this reason the period of germination at 3°C was extended for 24 weeks in order to facilitate full expression of germinability.

CONCLUSIONS

1. Germinability of full European hophornbeam seeds, separated out by floatation in water was greater after storage for 12 months at −3°C and seed moisture content of 7.5% than of seeds that were not stored in these conditions. This concerns results after a warm-followed-by-cold stratification

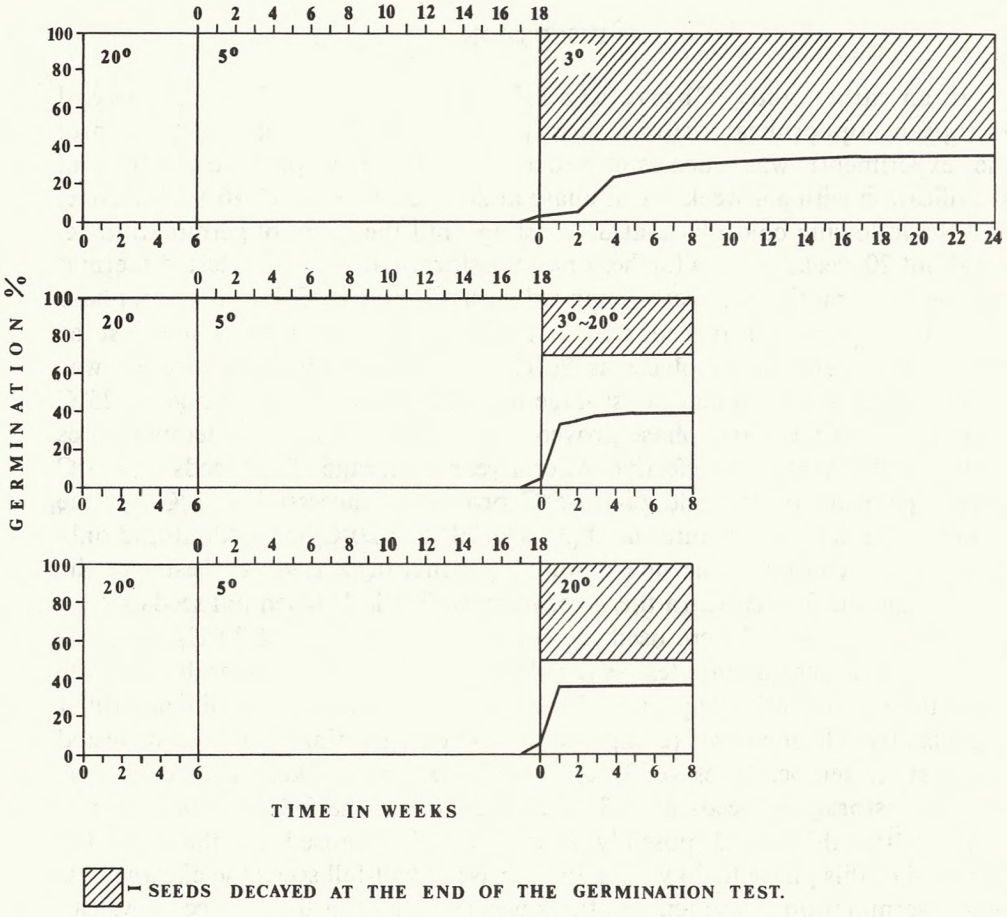

Fig. 3. *Ostrya carpinifolia* Scop. Course of germination of seeds after a warm-followed-by-cold stratification with the warm phase at 20°C and the cold phase at 5°C. The germination tests were conducted at a cyclically alternating temperature of 3°~20°C (A) and at constant temperatures 3°C (B) and 20°C (C)

with the warm phase at 20°C (2–6 weeks) and the cold phase at 3° or 5°C followed by germination at 3°, 3°~20°C and 20°C.

2. The duration of the warm phase of stratification (2–4 or 6 weeks) does not differentiate the germination of full seeds. Differences were also not observed regardless of the cold phase temperature, 3° or 5°C.

3. Germination temperature of 20°C proved as useful as the alternating 3°~20°C.

4. Full seeds floatated not only in water but also in ethanol, and not being washed in water after that, but dried to a moisture content of 7.6% and stored

for a year at 3°C had a germinative capacity significantly lower than for seeds discussed under conclusions 1–3, wich were not in contact with ethanol.

5. For half full seeds stored for one year at 3° and at −3°C the former assured higher germinability. After storage at −3°C all three temperatures of the warm stratification phase used, 15°, 20° and 25°C proved equally effective. Seeds stored at 3°C had higher germinability when the warm stratification phase was at 25°C than at 15° or 20°C. The most satisfactory germination test temperature for the half-full seeds was 3°~20°C.

RECOMMENDATIONS FOR PRACTICE

Full seeds should be separated from empty ones by floatation in water. A one year storage at −3°C does not decrease germination capacity, and even a slight increase is observable. Before sowing the seeds have to be subjected to a warm-followed-by-cold stratification with the warm phase at 20°C lasting 2–6 weeks and the cold phase at 3° or 5°C lasting about 20 weeks, i.e. till first germinating seeds appear.

LITERATURE

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Warunki ustępowania spoczynku i kiełkowania nasion chmielograbu europejskiego (*Ostrya carpinifolia* Scop.). Część II. Nasiona przechowywane przez 12 miesięcy

Streszczenie

Zdolność kiełkowania nasion chmielograbu europejskiego wzrasta po rocznym przechowywaniu w −3°C w porównaniu z nasionami przechowywanymi w −3°C przez miesiąc. Dotyczy to nasion poddanych stratyfikacji ciepło-chłodnej z fazą ciepłą w 20°C (2–6 tyg.) i chłodną fazą w 3° lub 5°C. Każda długość fazy ciepłej, tj. 2, 4 i 6 tygodni, była w równym stopniu skuteczna,

a temperatura fazy chłodnej 3°C tak samo efektywna jak 5°. Najkorzystniejszymi temperaturami kiełkowania dla tych nasion okazały się: zmienna 3°~20°C i stała 20°C. Zdolność kiełkowania, nasion pełnych wydzielonych po zbiorze przez kolejne spławianie w wodzie i etanolu, po rocznym przechowywaniu w −3°C i stratyfikacji była istotnie niższa od nasion spławianych tylko w wodzie. Korzystniejszą od −3°C temperaturą rocznego przechowywania nasion półpełnych okazała się temperatura 3°C.

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