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SILVER FIR SEEDS CONSERVATION

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1. INTRODUCTION

Regeneration is one of the fundamental processes during the forest's life, through which it is ensured the forest maintaining in space and time. The regeneration and extension of forest areas is an obligation and a priority for the national forestry sector (Romanian Forestry Code 2018). In the last period, the annual country-wide regenerated area has been approximately 28,000 ha, of which the artificially regenerated area represents 38%. Concerning artificial regeneration, coniferous seedlings occupy the majority share (57% of the total planted seedlings), Norway spruce being in the first place with 27%, followed by European silver fir with 5% (National Statistics Institute 2018). In our opinion, taking into consideration the ecological requirements of silver fir and the site conditions in the ecosystems of Romania, and also the economic value of this species, it would be required to use it to a greater extent than it has been used by now.

At an international level, one of the objectives of the Agenda UNO 2030 on sustainable development is to increase the forest areas and to reduce their fragmentation to ensure soil protection and the supply of forest products. In the context of climate change, maintaining forest productivity and increasing wood quality has become lately a major problem for the forestry domain. Climate change is leading to radical changes in the species geographical distribution, wood production and reproductive capacity (Hoffmann & Sgro 2011). EU regulations and policies regarding forest regeneration and carbon sequestration promote afforestation and reforestation with native species and local provenances as being one of the basic measures that can contribute to adapting and mitigating the effects of climate change on forest ecosystems.

Romania is under the influence of the temperate-continental climate and scenarios upon the effects of climate changes foreshadow the possibility of recording periods of excessive drought during the vegetation season, which cannot remain without consequences on the vitality and regeneration of the forest. Productivity and adaptability of tree populations are closely related to the genetic and qualitative characteristics of the forest reproductive material. Therefore, the production and use in reforestation of forest reproductive material with superior genetic traits will ensure the success of reforestation work and the capacity of the forest's ecosystems to adapt to environmental changes.

In this context, in addition to the aspects regarding the production of forest reproductive material with superior qualitative characteristics, the conservation of high-quality seeds is extremely important. Seeds conservation is necessary both in the short and long-term, given that most forest tree species do not have fructification yearly.

The European silver fir (*Abies alba* Mill.) is one of the most important species of the mountain ecosystems in Romania with multiple functions, including ecological, economic and soil protection. The periodicity of abundant fructifications of silver fir is between 4 to 5 years in seed stands and 2 to 3 years in seed orchards (Mihai, 2015). In Romania, there are 10 silver fir seed orchards with a total area of 85 ha and 3,344 ha seed stands in the selected category (Pârnuță et al., 2012). The seed production of silver fir seed orchards, in good fructification years, is approximately 300-400 kg/ha, and the viability percentage is on average 60%. Due to the very good fructification of silver fir seed orchards in Romania, the easiness of cones harvesting and the high quality of seeds, the forest reproductive material currently used in the reforestation works mostly comes from seed orchards. At the same time, given the high quality of silver fir seeds harvested from the seed orchards in our country, since 2010 the export of seeds from seed orchards managed by the National Forest Administration - Romsilva has increased.

Therefore, it is necessary to preserve the surplus of silver fir seeds in the good fructification years, at least until the next fructification. In Romania, a method for silver fir seeds conservation has not been established, and losses due to seed storage until the next spring can reach up to 50%. Because of this, literature in the country and existent technical guidelines recommend sowing the silver fir seeds in autumn, immediately after harvesting, to prevent the decrease in the percentage of germination (Haralamb 1967, Rubţov 1971).

The conservation conditions must ensure that the qualitative characteristics of the seed lot are maintained and that they will not be subject to depreciation during the storage period. The success of forest species seeds conservation depends on the knowledge of the factors that influence conservation, namely both genetic and initial qualitative characteristics of the seed lot (germination/viability, purity, moisture content, presence of pests, etc.), as well as of the storage conditions (temperature, type of packaging etc.) (Holmes & Buszewicz 1958, Barton 1961, Harrington & Jonnson 1970, Wang 1974). At the same time, in order to optimize the technical conservation procedures and prolong the longevity of seed preservation, it is important to determine and monitor the specific physiological processes that may occur in the mass of the seed lot.

Depending on these factors, forest species were classified as orthodox (Norway spruce, European larch, Scots pine), recalcitrant (sessile oak, common oak, horse chestnut tree) and intermediate (European silver fir, European beech) (Gosling 2007). The "orthodox" and "recalcitrant" categories were first introduced by Roberts (1973). The author defined the orthodox species as being those for which the seeds' moisture content could be lowered till 2 to 5%, and for which the longevity increased proportionally with the decreasing of the storage temperature. Recalcitrant species require high moisture content to maintain seed viability (40%), and the storage temperature cannot be lowered below -3 °C (Suszka 1971, 1974). Characteristic for intermediate species is that, being more sensitive to drying than orthodox species, seeds' moisture must be maintained within certain limits (10 to 12%), which leads to a shorter period of conservation (Hong et al. 1996, Gosling 2007).

More than 90% of the forest species from the temperate zone are orthodox. The silver fir is considered to be an intermediate species, which requires a higher moisture content to maintain seeds' viability (Barner 1975b). Also, silver fir seeds contain oils and resin in a high percentage (approximately 20%), which besides the beneficial role of protecting seeds from excessive drying or early germination can determine the rapid depreciation of seeds by breaking of resin vesicles and embryos contamination (Bouvarel & Lemoine 1958, Cermak 1987). Therefore, the main problems for the conservation of silver fir seeds are the following: 1) which are the qualitative characteristics of the seed lot that influence seed conservation, 2) what conditions must the conservation environment meet, 3) which would be the suitable conservation method, and 4) what would be the conservation period?

The main objective of this paper was to study the main factors that influence the viability of silver fir seeds during the conservation period and to establish an adequate method for the conservation of the silver fir seeds over a period of at least 3 to 4 years, as that is the fructification periodicity of silver fir seed orchards and seed stands in Romania.

The book is structured in five chapters and it is completed with a working protocol for silver fir seeds conservation. The first chapter presents the material and research method by describing the silver fir seed lots used, the environments tested for the conservation of silver fir seeds, the laboratory and statistical analyses carried out and the determined parameters. Chapter 2 presents the results concerning the genetic variability of some morphological and qualitative characteristics of the silver fir cones and seeds harvested from seed orchards and natural populations in Romania. Chapter 3 shows the results obtained during the experiments carried out in the period 2010 - 2019, in order to determine the silver fir seeds conservation method, by testing various storage conditions.

This chapter also describes pre-treatments tested to stimulate the germination capacity of the stored seeds, emergence percentage of stored seeds in the nursery compared to fresh ones and characteristics of seedlings obtained from conserved seeds. Chapter 4 presents a series of recommendations concerning harvesting and processing of silver fir cones, extraction of seeds from cones, drying and processing of silver fir seeds. We consider that these recommendations are very useful for obtaining high-quality seeds both in terms of conservation and improving the technology for producing silver fir seedlings in nurseries. The final chapter presents the conclusions drawn from the analysis of the research outcomes.

The book is addressed to forest engineers who work in the field of forest regeneration, researchers and anyone interested in the breeding and culture of this species in nurseries.

We give thanks to National Forest Administration-Romsilva for funding the research project: "Conservation of silver fir seeds" (research cycle 2017 to 2019) and to the Sibiu and Covasna Forest Counties for the support given during the research.

Also, we give thanks to the colleagues from the Forest Genetic Laboratory in Bucharest ("Marin Drăcea" National Institute for Research and Development in Forestry) for their contribution to the accomplishment of the scientific activities of the project and our colleague Eliza-Maria Cosma for the English review and technical writing work.

Also, we wish to thank Dr Monika Konnert (Institute for Seeds and Seedlings, Bavaria) and University-Professor Neculae Șofletea (Transilvania University of Brasov) for the useful pieces of advice and appreciation reports elaborated for the publication of this book.

The authors' contribution is as follows: Georgeta MIHAI established the working method and carried out the researches in chapters: 2, 3 and 4. Alin-Mădălin ALEXANDRU has implemented the method of assessing the seeds' respiration using the device „Insect Respiration Chamber" and carried out the analyses regarding the respiration of silver fir seeds. Both authors wrote chapter 5.

2. MATERIAL AND RESEARCH METHOD

2.1. Research material

The research material consists of seed lots harvested from silver fir natural populations (forest genetic resources, seed stands or regular stands) as well as from seed orchards installed in Romania (Table 1). The sampled populations come from the main geographical regions, being located in 10 regions of provenance (Pârnuţă et al. 2010).

From seed orchards, the cones were harvested in 4 years with very good fructifications for silver fir in Romania (2010, 2013, 2015 and 2018), but also in 2016, when fructification was very low. These seed lots were subject of both export and domestic sale by forestry units. The harvesting of cones in natural populations was performed only in the year 2010.

In order to evaluate the genetic variability of silver fir seeds and cones characters, the study material consisted in 12 natural populations (Avrig, Marginea, Remeţi, Rusca Montană, Bicaz, Sinaia, Dobra, Cozia, Anina, Strâmbu Băiuţ, Comandău and Gârda) and 4 seed orchards (Avrig, Tălișoara, Baia Sprie and Gârcina) (Table 1). In each population 20 trees of the dominant or co-dominant categories were sampled so that the distance between them was more than 30 meters. The cones were harvested by climbing, 20 cones being collected from each mother tree, constituting different seed lots whose genetic identity was maintained during the whole experiment. In seed orchards 20 clones were sampled to cover all provenance regions of the plus trees included in the composition of these seed orchards. A total of 320 silver fir open-pollinated families were established and were sown in the nursery in autumn 2010.

The research material used to determine the method for silver fir seeds conservation consisted of 62 seed samples, belonging to 16 seed lots harvested from 7 seed orchards (Avrig, Tălişoara, Târgovişte, Baia Sprie, Gârcina, Văliug and Dumitreşti) in 5 years of fructification (2010, 2013, 2015, 2016, 2018).

Table 1. Silver fir populations and seed orchards included in the study

No. ctr.	Forest District	Provenance region	Type of basic material	Lat. N	Long. E	Alt. (m)
1	Avrig	C1	Seed orchard	45.66	24.44	630
2	Tălişoara	B1	Seed orchard	45.86	25.70	760
3	Baia Sprie	A1	Seed orchard	47.82	23.67	1070
4	Gârcina	A2	Seed orchard	47.02	26.52	445
5	Târgovişte	C2	Seed orchard	44.56	25.23	340
6	Văliug	D1	Seed orchard	45.13	22.00	620
7	Dumitreşti	B2	Seed orchard	45.38	27.01	300
8	Avrig	C1	Selected source, FGR[1]	45,65	24,49	840
9	Marginea	A2	Selected source, FGR	47.82	25.70	690
10	Remeţi	E2	Selected source	46.74	22.69	1180
11	Rusca Montană	D2	Selected source, FGR	45.66	22.39	1050
12	Bicaz	A2	-	46.84	26.03	980
13	Sinaia	B2	-	45.31	25.55	1130
14	Dobra	C1	FGR	45.68	22.51	1075
15	Cozia	C2	Selected source, FGR	45.32	24.37	1220
16	Anina	D1	Selected source, FGR	45.07	21.89	725
17	Str. Băiuţ	A1	Selected source, FGR	47.62	24.01	770
18	Comandău	B1	Selected source, FGR	45.71	26.31	1050
19	Gârda	E3	-	46.46	22.90	760

1)Selected source: basic material from selected category, FGR: forest genetic resource

Photo 1. Silver fir genetic resource from Avrig Forest District

2.2. Seed lots sampling and laboratory analyses

Seed lots sampling and laboratory analyses for seed samples which were to be stored for conservation were carried out in accordance with the ISTA Rules editions 2010, 2013 and 2015, and to the national Standards SR 1808 / 2004 and SR 1908 / 2004.

In the case of seed lots intended for export, sampling was performed by a sampler authorised by "Marin Drăcea" National Institute for Research and Development in Forestry, by taking several elementary samples randomly, which formed the composite samples and which were subsequently submitted for analysis to the Seed Laboratory from Bucharest. In the case of seed lots intended for internal use, sampling was performed by the staff responsible for this activity in each forest district, according to national standard SR 1808/2004.

The laboratory analyses consisted in determining the following seeds morphological and physiological parameters: the purity (%), the viability (%), the weight of 1000 seeds (g), the moisture content (%) and the respiration. The purity analysis was carried out based on two analytical subsamples constituted with the help of the soil divider. Viability was evaluated by the tetrazolium test, based on the average of four repetitions (each repetition containing 100 seeds). This method has two advantages compared to the germination analysis, it is faster and is not influenced by the dormant phenomenon. The weight of 1000 seeds has been calculated based on the average of 8 repetitions (in accordance with ISTA Rules 2015 and SR 1908 / 2004).

In addition to these parameters, for each seed sample, there have been determined: moisture content, initially and during the conservation period, and seeds' respiration. Moisture analysis was carried out according to the method described in SR 1908/2004 and ISTA Rules 2015, based on two analytical subsamples that were dried in the oven at a constant temperature of 103 ºC for 17 hours.

For the measurement of the seeds' respiration a new method, different from the known ones of Warburg and Bozsen – Jensen, was used to assess seeds respiration, namely, using an 'Insect Respiration Chamber' device fitted to the CIRAS 2. Although this device has only been used for insect's respiration measuring so far, the results obtained by us show that this device can also be used for measuring forest seeds respiration, provided that they have small or medium sizes. The rate of silver fir seeds respiration (RRS) has been determined using the following formula:

RRS (µgCO_2/g/min) = CO_2d (ppm) x flux (ml/min) x 44.0 g/22400 ml x 10E6 (parts/ppm) x 10E6 (µg/g) / weight of the seeds sample (g)

Where: 44.0 g/22400 ml = density of CO_2 at standard temperature and pressure, CO_2d = difference of CO_2, flux (ml/min) = tube flow rate.

2.3. Conservation environments

In order to determine the most suitable conservation method for silver fir seeds, five conservation environments were established. The five conservation environments and seed samples stored in each of them are described in Table 2. The first conservation environment (M1) was arranged in the spring of the year 2011, in a refrigerating room of the Sibiu Forest County. In 2017, another 4 conservation environments were established: 2 refrigerating rooms at Brețcu Forest District, Covasna Forest County (M2 and M3), a refrigerator in the Seed Laboratory in Bucharest (M4) and the fifth environment consists in the Seed Conservation Centre in Brașov (M5). The temperature of the 5 conservation environments has been between -7 ºC and +5 º C, with a variation range of ±1 ºC. Only for M4 environment the temperature variation has been higher (±2 ºC) (Table 2).

Table 2. Tested conservation environments

No. crt.	Medium	Temperature of conservation (ºC)	Administrative location	No. of stored samples	Year of fructification
1	M1	-5 (±1ºC)	Sibiu Forest County	18	2010, 2013, 2015, 2016, 2018
2	M2	-3 (±1ºC)	Breţcu Forest District	11	2015, 2016, 2018
3	M3	-7 (±1ºC)	Breţcu Forest District	11	2015, 2016, 2018
4	M4	+5 (±2ºC)	INCDS, Bucharest	11	2015, 2016, 2018
5	M5	+3 (±1ºC)	INCDS, Brașov Station	11	2015, 2016, 2018

In M1 environment 19 seeds samples have been stored, starting with 2010 fructification, until 2018. In the other four environments there have been stored 11 seed samples from the fructifications of the years 2015, 2016 and 2018.

Polyethylene and raffia bags have been used for packaging. All seed samples were packed in raffia bags which then have been placed in polyethylene bags, with one exception, Avrig 2015 seeds sample, which have been packed only in raffia bag. The polyethylene bags have been hermetically sealed to prevent moisture exchange with the conservation environment (Pasquini et al., 2012). The seeds thus packed have been stored in the refrigerating rooms.

2.4. Data processing and statistical analysis

In order to investigate the genetic variability of morphological and qualitative characters of silver fir seeds and cones harvested in natural populations and seed orchards, the following statistical parameters have been initially calculated: average (x), variance (s^2), standard deviation (s), coefficient of variation (CV), minimum and maximum values.

Multiple variance analysis with the following mathematical model (Nanson 2004) has been used to detect the effect of the genetic factor in the total phenotypic variance:

$$Y_{kijl} = \mu + P_k + R_i + F_j + e_{ijkl}$$

where: μ = the average on experiment; P_k = the effect of k^{th} populations; R_i = the effect of ith repetitions; F_j = the effect of j^{th} families; e_{kijl} = the error.

Also, Pearson correlation coefficients have been calculated among the studied characters, as well as correlations between characters and the ecological gradients of the place of origin. Multiple linear regressions have been performed to highlight the most important factors that influence silver fir seeds conservation. Data obtained through observation and field measurements has been statistically processed using the SPSS program (version 19).

Photo 2. Silver fir genetic resource from Strâmbu Băiuț Forest District

Photo 3. Fructification in the Avrig seed orchard

3. GENETIC VARIABILITY OF SOME MORPHOLOGICAL AND QUALITATIVE CHARACTERS OF SILVER FIR CONES AND SEEDS

The statistical analysis of silver fir cones and seeds characters, at the level of populations and seed orchards included in the 2010 experiment is presented in Table 3.

Table 3. Analysis of variance for some morphological and qualitative characteristics of silver fir seeds and cones harvested from natural populations and seed orchards, in the year 2010

Source of variation	DF	Variance (s^2)			
		Cones Length	Cones Diameter	Weight of 1000 seeds	Emergence percentage
Natural populations (P)	11	11.219***	32.819**	63.760***	1434.108***
Seed orchards (O)	3	18.915***	62.521**	31.313***	921.332***
Populations vs. Orchards	1	144.454***	2.834	399.920***	10409.510***
Families in total (P + O)	319	77.742***	93.251***	7.087***	292.376***
Families in natural populations	239	89.853***	85.617***	5.948***	251.826***
Families in seed orchards	79	35.334***	36.169***	5.561***	180.338***
Error	110	1.454	12.709	0.789	143.519

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The results highlighted a significant genetic variability both at inter- and intra-population levels (between open-pollinated families of selected trees in natural populations). At the same time, for all considered characteristics, there were also statistically ensured differences among the 4 silver fir seed orchards included in the experiment, and also among the clones from seed orchards.

Differences between seeds and cones characteristics of the two seed source categories, populations vs. seed orchards, were highly significant ($p < 0.001$) except for cones diameter. The main statistical parameters of seeds' and cones' characteristics for the analysed seed orchards and populations are shown in Tables 4 and 5.

Table 4. Main statistical parameters for characteristics of silver fir seeds and cones harvested in seed orchards in the year 2010

Trait	Seed orchard	Average	Minimum value	Maximum value	Variance	Coefficient of variation
Weight of 1000 seeds (g)	**Seed orchards - total**	**68.88**	**38.80**	**97.60**	**138.99**	**17.20**
	Avrig seed orchard	68.40	54.53	81.60	69.25	12.20
	Tălişoara seed orchard	68.43	38.80	97.60	231.73	22.30
	Baia Sprie seed orchard	76.98	59.60	95.40	91.99	12.50
	Gârcina seed orchard	61.72	46.20	73.90	61.45	12.70
Cones length (cm)	**Seed orchards - total**	**15.51**	**12.48**	**19.06**	**3.66**	**12.40**
	Avrig seed orchard	15.79	13.55	18.53	3.01	11.00
	Tălişoara seed orchard	15.21	12.48	19.06	4.65	14.20
Cones diameter (mm)	**Seed orchards - total**	**31.75**	**25.89**	**38.01**	**14.64**	**12.10**
	Avrig seed orchard	33.28	26.87	38.01	14.90	11.60
	Tălişoara seed orchard	30.05	25.89	34.37	9.99	10.60

Trait	Seed orchard	Average	Minimum value	Maximum value	Variance	Coefficient of variation
Emergence percentage (%)	**Seed orchards - total**	**43.37**	**1.00**	**72.00**	**406.29**	**46.50**
	Avrig seed orchard	59.36	45.00	68.00	63.05	13.40
	Tălişoara seed orchard	40.00	4.00	72.00	718.22	67.00
	Baia Sprie seed orchard	47.00	34.00	60.00	61.96	16.50
	Gârcina seed orchard	24.70	1.00	47.00	221.12	60.20

Table 5. Main statistical parameters for characteristics of silver fir seeds and cones harvested in natural populations, in the year 2010

Trait	Population	Average	Minimum value	Maximum value	Variance	Coefficient of variation
Weight of 1000 seeds (g)	**Populations total**	**55.94**	**30.30**	**91.30**	**145.39**	**21.6**
	Avrig	58.53	37.50	85.00	150.25	21.0
	Marginea	49.85	40.00	67.10	45.44	13.6
	Remeţi	44.58	30.40	54.03	31.00	12.5
	Rusca Montană	58.65	43.78	69.90	55.41	12.7
	Bicaz	54.57	44.00	72.18	56.82	13.9
	Sinaia	62.98	44.83	85.90	141.04	18.9
	Dobra	66.89	53.10	91.30	113.12	15.9
	Cozia	41.65	30.30	56.35	51.92	17.4
	Anina	66.15	57.03	82.93	55.49	11.3
	Strâmbu Băiuţ	66.00	49.30	100.48	151.90	18.7
	Comandău	45.21	37.00	57.50	31.31	12.4
	Gârda	56.65	39.83	71.20	63.95	14.2

Trait	Population	Average	Minimum value	Maximum value	Variance	Coefficient of variation
Cones length (cm)	**Populations -total**	**12.51**	**9.26**	**16.28**	**2.02**	**11.4**
	Avrig	12.68	10.41	14.89	1.79	10.6
	Remeţi	11.32	10.42	13.21	0.84	8.1
	Rusca Montană	13.94	12.13	15.62	0.98	7.1
	Bicaz	12.10	10.61	14.20	1.45	10.0
	Sinaia	13.31	12.28	15.25	1.12	8.0
	Dobra	12.31	10.35	14.07	1.10	8.6
	Cozia	11.20	10.33	12.40	0.43	5.8
	Anina	14.06	12.47	14.94	0.61	5.6
	Strâmbu Băiuţ	14.13	12.95	16.28	2.16	10.4
	Comandău	11.21	9.26	12.59	0.89	8.4
	Gârda	12.34	11.27	13.78	0.61	6.4
Cones diameter (mm)	**Populations -total**	**32.17**	**8.21**	**38.66**	**14.68**	**12.0**
	Avrig	31.71	27.19	35.48	9.91	10.0
	Remeţi	31.11	28.80	36.77	6.84	8.5
	Rusca Montană	30.04	8.21	35.37	63.07	26.5
	Bicaz	28.37	23.88	34.80	13.33	12.9
	Sinaia	34.27	30.80	37.56	5.69	7.0
	Dobra	32.61	30.07	35.79	4.51	6.5
	Cozia	32.11	26.57	35.96	6.84	8.2
	Anina	34.08	30.87	37.85	5.61	7.0
	Strâmbu Băiuţ	33.87	31.64	38.66	10.58	9.6
	Comandău	33.21	29.99	36.95	5.64	7.2
	Gârda	33.58	28.61	36.66	5.99	7.3

Trait	Population	Average	Minimum value	Maximum value	Variance	Coefficient of variation
Emergence percentage (%)	**Populations -total**	**19.33**	**0.00**	**64.00**	**369.37**	**99.0**
	Avrig	11.85	1.00	38.00	201.64	120.0
	Marginea	3.67	0.00	12.00	16.82	111.8
	Remeţi	17.20	2.00	40.00	173.07	76.6
	Rusca Montană	34.72	0.00	64.00	695.29	76.0
	Bicaz	3.25	0.00	25.00	35.16	182.5
	Sinaia	4.32	0.00	33.00	91.36	221.3
	Dobra	39.56	9.00	64.00	281.78	42.5
	Cozia	27.00	6.00	43.00	207.00	53.3
	Anina	22.62	1.00	56.00	471.76	96.1
	Strâmbu Băiuţ	40.44	30.00	51.00	70.03	20.7
	Comandău	23.06	0.00	36.00	117.56	47.0
	Gârda	24.99	0.67	45.66	204.53	57.3

It can be observed that for all analysed characters, seed orchards obtained higher average values than populations. The amplitude of variation of seeds' characteristics has been higher in the stands and the coefficients of variation have been smaller in seed orchards, which indicates the increased stability of qualitative characteristics for seeds obtained in seed orchards.

The weight of 1000 seeds varied between 41.65 g to 66.89 g in the Cozia and Dobra populations, respectively. In the case of seed orchards, the weight of 1000 seeds varied between 76.98 g to 61.72 g for Baia Sprie and Gârcina seed orchards, respectively. Also, the variation among the open-pollinated families of trees selected in stands has been higher compared to the variation among the open-pollinated families of plus trees from seed orchards, ranging from 30.30 g to 91.30 g in the case of stands and from 38.80 g to 97.60 g in the case of seed orchards.

Regarding the size of silver fir cones, the ones harvested in seed orchards have been larger than those from populations. Amongst the silver fir populations, only Anina, Strambu Baiut and Sinaia have had cones that had sizes close to those from seed orchards. The cones' length has been positively correlated and highly significant to the weight of 1000 seeds. Both the cones' length and weight of 1000 seeds have been negatively correlated to the altitude of origin of the populations, indicating a clinal variation (Table 6).

Table 6. Correlations among morphological characters of cones and seeds from silver fir populations and geographical gradients of origin location

Character	Cones length	Cones diameter	Altitude	Lat. N	Long. E
Weight of 1000 seeds	0.833**	0.333	-0.578*	0.003	-0.337
Cones length	-	0.258	-0.581*	0.012	-0.366
Cones diameter	-	-	-0.314	-0.226	-0.126

* p < 0,05; ** p < 0,01; *** p < 0,001.

As regards the emergence percentage of seeds sown in the nursery, the differences between stands and seed orchards have been extremely large. Thus, the value of the coefficient of variation has been 99% for stands and 46% for seed orchards, and the average value has been x = 43.37% and x = 19.33% for seed orchards and stands, respectively. Seed orchards have registered a better emergence percentage than seed stands, the best results being recorded in the case of Avrig seed orchard (x=59.36%). The Avrig seed orchard also displayed higher homogeneity, obtaining the lowest coefficient of variation (CV = 13%), compared to the Tălișoara (CV = 67%) and Gârcina (CV = 60%) seed orchards, which showed higher variability for this trait. For studied populations of silver fir, the best emergence has been recorded by the Strâmbu Băiuț population (x = 40%) and the lowest by Bicaz and Marginea populations (x = 3%). This extremely large variability of emergence percentage for seeds harvested from populations compared to those harvested in seed orchards represents a consequence of the pollination process. Even in the good flowering years, due to the high density of trees, pollination within populations is made with difficulty, leading to a higher percentage of empty seeds. In the cases of Tălișoara and Gârcina seed orchards the high value of the variation coefficients can be explained by the existence of phenological differences among the component clones, differences observed during the technical assistance work carried out in these seed orchards, that have determined the non-synchronization of the anthesis with the female flowers' receptivity during the pollination process (Mihai 2015).

Photo 4. Tălișoara silver fir seed orchard (Covasna Forest County)

Photo 5. Târgoviște silver fir seed orchard (Dâmbovița Forest County)

No significant correlation has been found between the weight of 1000 seeds and the percentage of seeds' emergence in the nursery. However, there was a negative significant correlation between the emergence percentage and the growth of seedlings in the early years in the nursery (Table 7).

Table 7. Correlations among weight of 1000 seeds, emergence percentage and growth characters of silver fir seedlings in nursery at 2 years old

Character	Emergence percentage	Diameter at root collar	Height
Weight of 1000 seeds	0.247 / 0.191	0.470* / 0.444**	0.403* / 0.539**
Emergence percentage	-	-0.672*** / -0.156	0.396* / 0.131

At the numerator: the correlation coefficients determined in seed orchards; at denominator: the correlation coefficients determined in natural populations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Hence, a higher emergence rate resulted in smaller collar diameters of seedlings, as a result of insufficient nutrition space. The seeds' weight has positively influenced the growth traits of the seedlings in the first 2 years of vegetation. Results confirm that the large seeds with high qualitative characteristics produce more vigorous seedlings due to higher amounts of nutrients in endosperm, this being the effect of the maternal factor. Similar results have been obtained for other species too: *Picea abies* (Skrøppa & Torleiv 1990), *Pseudotsuga menziesii* (Sorensen & Campbell 1993), *Pinus strobus* (Parker et al. 2006), *Castanea sativa* (Cicek & Tilki 2007), *Larix decidua* (Gorian et al. 2007), *Pinus Densata* (Xu et al. 2016).

In addition to this genetic determination, the values of the qualitative parameters of the seed lots that come from seed orchards have varied with the fructification years. Analysing the weight of 1000 seeds and the viability of the seed lots harvested from 7 seed orchards, in 3 years of fructification, we found that the lowest values have been obtained in 2016, a low fructification year for silver fir in Romania. Even in very good fructification years, due to the different genetic compositions of the seed orchards, significant differences have been recorded amongst seed lots that came from seed orchards in terms of the weight of 1000 seeds (Fig.1). The weight of 1000 seeds, determined in this case at the level of a whole lot, varied considerably amongst the analysed seed orchards and had values between 59 to 85 g.

The maximum value for the weight of 1000 seeds has been obtained in the

Târgoviște seed orchard, in all the considered fructification years (85–68 g), and the minimum value in the Avrig seed orchard in 2018 (59 g). Moreover, besides this genetical variability, the climatic factors had a substantial influence on the seeds' weight, the highest values being recorded, in all seed orchards, in 2013, one of the rainiest years in Romania over the last 40 years (www.meteoromania.ro).

As far as seeds' viability is concerned, no significant differences among seed orchards have been obtained. The highest percentage of viability has been achieved in the Baia Sprie seed orchard, in 2015 (67%), followed by Tălișoara (65% in 2013) and Avrig (64% in 2013) seed orchards. The lowest viability has been constantly achieved in the Târgoviste seed orchard (39–51%). Therefore, there is no significant correlation between the weight and the seeds' viability. According to the working technique described in the standards mentioned in the introduction, seeds' viability has been evaluated based on the viability of the embryos and the health of the endosperm of the seeds, and it is not influenced by the weight of the seeds as it has been observed in the case of technical germination (Baldwin 1942, Dunlap & Barnett 1984, Sabor 1984, Muhle et al. 1985). According to the results of these authors, the seeds' weight and size influence significantly the seed' germination. The viability analysis is a faster and more rigorous method compared to the germination method and it is recommended by the ISTA Rules, particularly for silver fir.

The results obtained in other countries show much lower values for the qualitative parameters of silver fir seeds compared to those obtained by us in the presented experiment. The data published on the Bavarian State Forest Institute website (www.lwf.bayern.de › biodiversitaet) for local provenances of silver fir show values for the weight of 1000 seeds up to 45 g and technical germination between 35–45%. Data published by the Seeds and Seedlings Institute in Teisendorf (AWG) regarding the characteristics of silver fir seeds from the southern Germany, also show values for the weight of 1000 seeds between 35–50 g. In Bosnia and Herzegovina, Bailian (2013) obtained significant variability among both the populations and sub-populations of the silver fir regarding the morphological and physiological characteristics of the seeds. For the weight of 1000 seeds, there have been obtained values between 32 to 76 g, and the germination rate varied from 4 to 60%. Similar results have been obtained by Gagov (1973) in Bulgaria and Laffers (1979) in Slovenia, with the germination rate values ranging between 5–70% and for seeds' weight values between 34–82 g and 33–76 g, respectively. In Poland, Skrzyszewska & Chłanda (2009) obtained values below 55 g for seeds' weight and 44% viability for seeds, specifying that these values represent the average values at the country level.

Photo 6. Gârcina silver fir seed orchard (Neamț Forest County)

4. CONSERVATION OF SILVER FIR SEEDS

4.1. Conservation environments and qualitative parameters of stored seed lots

This chapter presents the results obtained by preserving 62 silver fir seed samples, belonging to 16 lots harvested in 5 fructification years (2010, 2013, 2015, 2016, 2018), in the 5 refrigerated chambers outlined in the previous chapter. The values of the qualitative parameters of the seed lots initially and during the conservation period are presented in Table 8.

Table 8. Qualitative parameters for silver fir seed samples stored in the five conservation environments, in period 2011 - 2019

No crt.	Seed orchard	Year of harvest	Year of storage	Qualitative parameters at the beginning of conservation			Qualitative parameters during conservation period			Percentage of viability maintenance %
				Purity %	Moisture content %	Viability %	Viability 2018 %	Moisture content 2019 %	Viability 2019 %	
M1 (-5°C)										
1	Avrig	2010	2011	92.8	6.54	59	57	-	-	97
2	Avrig	2010	2011	97.0	6.71	61	59	-	-	97
3	Avrig	2010	2011	96.7	6.66	51	50	-	-	98
4	Avrig	2013	2013	98.1	8.19	64	56	8.71	39	61
5	Avrig	2015	2015	97.2	11.94	54	52	9.39	28	52
6	Avrig	2016	2016	97.1	16.52	44	36	16.78	6	14
7	Avrig	2018	2018	98.1	16.82	63	63	17.61	59	94
8	Tălişoara	2013	2013	96.6	9.86	65	56	9.42	38	58
9	Tălişoara	2015	2015	93.0	11.30	61	54	11.81	41	67
10	Tălişoara	2018	2018	93.3	15.42	62	62	14.46	58	94
11	Târgoviște	2013	2013	97.8	8.80	47	42	8.15	36	77
12	Târgoviște	2015	2015	98,0	10,53	52	49	10,36	32	62

No crt.	Seed orchard	Year of harvest	Year of storage	Qualitative parameters at the beginning of conservation			Qualitative parameters during conservation period			Percentage of viability maintenance %
				Purity %	Moisture content %	Viability %	Viability 2018 %	Moisture content 2019 %	Viability 2019 %	
13	Târgoviște	2018	2018	95.8	16.62	39	39	15.22	21	54
14	Baia Sprie	2013	2013	97.3	8.63	39	29	10.51	24	62
15	Baia Sprie	2018	2018	83.8	15.51	58	58	14.95	53	91
16	Gârcina	2018	2018	96.0	25.55	58	58	25.44	54	93
17	Văliug	2018	2018	90.0	15.80	50	50	15.40	33	66
18	Dumitreşti	2018	2018	91.5	11.20	39	39	11.19	32	82
M2 (-3°C)										
19	Avrig	2015	2017	97.2	11.94	54	43	12.31	22	41
20	Avrig	2016	2017	97.1	16.52	44	33	17.19	6	14
21	Avrig	2018	2018	98.1	16.82	63	63	17.18	59	94
22	Târgovişte	2015	2017	98.0	10.53	52	42	10.71	30	58
23	Târgoviște	2018	2018	95.8	16.62	39	39	15.99	18	46
24	Tălişoara	2015	2017	93.0	11.30	61	52	11.76	37	61
25	Tălişoara	2018	2018	93.3	15.42	62	62	15.68	60	97
26	Baia Sprie	2018	2018	83.8	15.51	58	58	15.57	52	90
27	Gârcina	2018	2018	96.0	25.55	58	58	27.45	50	86
28	Văliug	2018	2018	90.0	15.80	50	50	14.86	34	68
29	Dumitreşti	2018	2018	91.5	11.20	39	39	10.25	31	79
M3 (-7°C)										
30	Avrig	2015	2017	97.2	11.94	54	44	12.84	32	59
31	Avrig	2016	2017	97.1	16.52	44	31	17.57	0	0
32	Avrig	2018	2018	98.1	16.82	63	63	17.75	55	87
33	Târgoviște	2015	2017	98.0	10.53	52	49	11.46	33	63
34	Târgoviște	2018	2018	95.8	16.62	39	39	15.59	17	44
35	Tălişoara	2015	2017	93.0	11.30	61	61	12.02	41	67
36	Tălişoara	2018	2018	93.3	15.42	62	62	15.17	57	92
37	Baia Sprie	2018	2018	83.8	15.51	58	58	14.99	49	84
38	Gârcina	2018	2018	96.0	25.55	58	58	27.07	51	88

No crt.	Seed orchard	Year of harvest	Year of storage	Qualitative parameters at the beginning of conservation			Qualitative parameters during conservation period			Percentage of viability maintenance %
				Purity %	Moisture content %	Viability %	Viability 2018 %	Moisture content 2019 %	Viability 2019 %	
39	Văliug	2018	2018	90.0	15.80	50	50	15.28	32	64
40	Dumitreşti	2018	2018	91.5	11.20	39	39	10.69	31	79
M4 (+5°C)										
41	Avrig	2015	2017	97.2	11.94	54	40	11.30	6	11
42	Avrig	2016	2017	97.1	16.52	44	9	7.51	0	0
43	Avrig	2018	2018	98.1	16.82	63	63	17.75	50	79
44	Târgovişte	2015	2017	98.0	10.53	52	45	7.27	23	44
45	Târgovişte	2018	2018	95.8	16.62	39	39	11.28	16	41
46	Tălişoara	2015	2017	93.0	11.30	61	47	7.96	17	28
47	Tălişoara	2018	2018	93.3	15.42	62	62	11.00	42	68
48	Baia Sprie	2018	2018	83.8	15.51	58	58	12.98	28	48
49	Gârcina	2018	2018	96.0	25.55	58	58	22.91	37	64
50	Văliug	2018	2018	90.0	15.80	50	50	13.31	25	50
51	Dumitreşti	2018	2018	91.5	11.20	39	39	9.30	25	64
M5 (+3°C)										
52	Avrig	2015	2017	97.2	11.94	54	36	11.90	0	0
53	Avrig	2016	2017	97.1	16.52	44	15	15.60	0	0
54	Avrig	2018	2018	98.1	16.82	63	63	16.52	51	81
55	Târgovişte	2015	2017	98.0	10.53	52	45	11.81	22	45
56	Târgovişte	2018	2018	95.8	16.62	39	39	16.10	11	28
57	Tălişoara	2015	2017	93.0	11.30	61	53	12.00	20	32
58	Tălişoara	2018	2018	93.3	15.42	62	62	14.99	54	87
59	Baia Sprie	2018	2018	83.8	15.51	58	58	15.23	50	86
60	Gârcina	2018	2018	96.0	25.55	58	58	26.59	42	72
61	Văliug	2018	2018	90.0	15.80	50	50	14.87	31	62
62	Dumitreşti	2018	2018	91.5	11.20	39	39	10.82	18	46

Table 9. Analysis of variance for qualitative parameters of silver fir seeds after four years and one year of conservation

Source of variation	DF	Variance (s^2)			
		Purity	Initial viability	Viability after four years	Viability after one year
Conservation environment	4	-	-	5.424**	17.383***
Seed orchard	6	4.017	92.016	625.471**	1078.400***
Year of fructification	2	3.028	2.100	2641.571**	-
Error	110	5.490	28.000	180.000	41.214

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

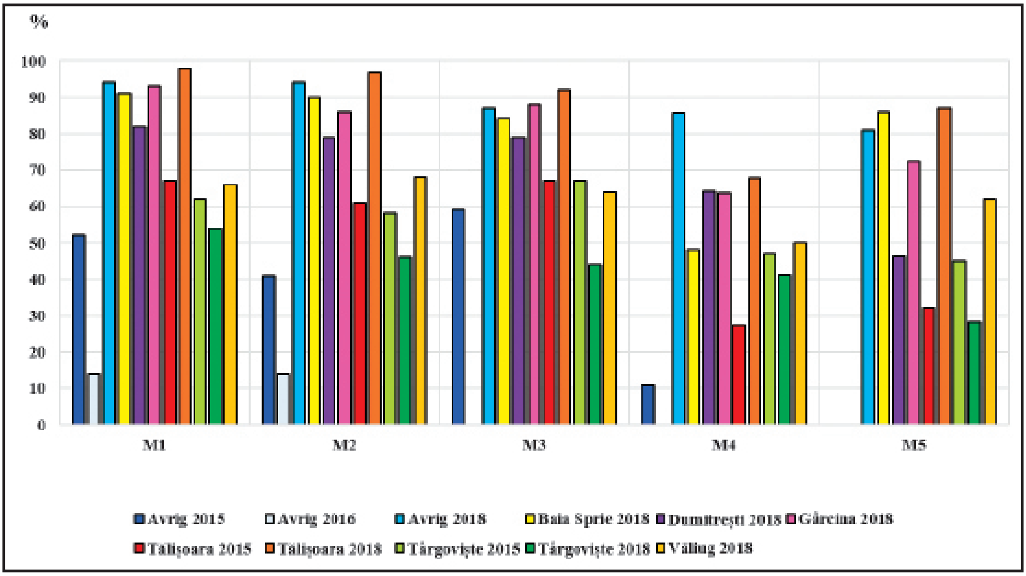

Fig. 1. Percentage of viability maintenance for silver fir seed lots, harvested in years 2015, 2016 and 2018 in seed orchards, in the five conservation environments

At the level of the whole experiment, after one year and four years of conservation, respectively, the viability of the seeds varied significantly depending on the conservation environment, fructification year and seed orchard (Table 9). Overall, the highest percentages of viability have been recorded in the M1 environment (Sibiu), at a conservation temperature of -5 °C, both after four years and one year of conservation.

The seed lot harvested from the Avrig seed orchard in 2016, which has been a very poor fructification year for silver fir, recorded the highest viability losses, across all conservation environments. Amongst all seed lots harvested in 2018, the ones from Targoviste and Valiug seed orchards had the lowest percentage of maintaining the viability, in all the environments.

The results obtained by the seed lots in each conservation environment are shown in Table 8. In M1 environment, where the storage temperature has been -5 °C, 18 seed samples have been stored: 7 samples from the year 2018 and 11 samples harvested in 2010, 2013, 2015 and 2016 (Fig. 2).

Analysis of the results has showed a loss of viability of only 2% for the 3 seed samples belonging to the 2010 fructification and preserved for 8 years (2011–2018) in this store. For the seed samples harvested in 2013 and preserved for 5 years (2013–2018), the viability percentages decreased from 23% to 48%. For the seed samples from 2018, the viability losses have been 6–46%. The highest viability losses have been recorded by the Avrig 2016 sample (86%). The substantial losses recorded by Avrig 2015 (48%) and Targoviste 2018 (46%) samples have been due to the packaging type (in the first case) and the seed processing (in the second case). Thus, in the case of the Avrig 2015 sample, the seeds had been packaged only in raffia bags, without being placed in polyethylene bags afterwards. The moisture exchange with the atmosphere of the refrigerating room led to the depreciation of the seeds stored in this way. Also, processing the seeds by removing seed wings, as is has been done in the case of the Targoviste 2018 sample, caused the breaking of the resin pockets, leading to the reduction of the viability of the embryos and even their death.

A highly significant regression has been obtained between the viability of the conserved seeds and the initial viability, as well as with the seed moisture content. Thus, the viability of the seeds during the conservation period is positively and significantly correlated with the viability at the beginning of the conservation and negatively and highly significantly correlated with their moisture content (Table 10).

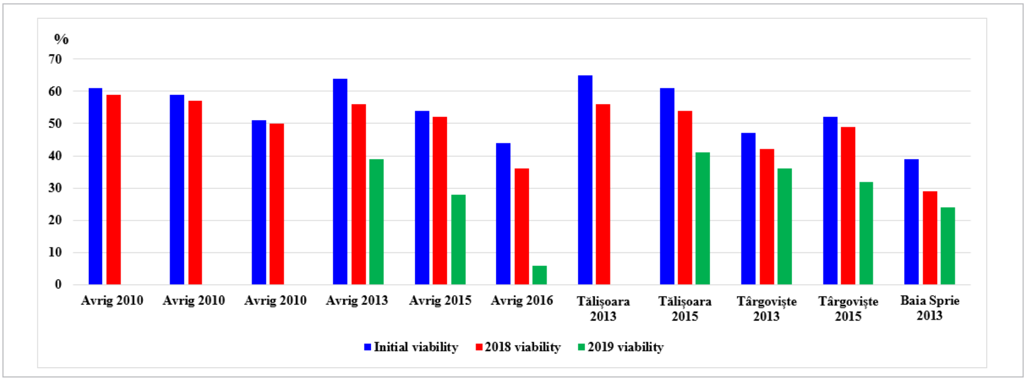

Fig. 2. Variation of viability for silver fir seed lots from fructification years 2010, 2013, 2015 and 2016, conserved in M1 environment (-5° C, Sibiu)

Table 10. Influence factors and regression equation for viability of silver fir seeds conserved in M1 environment, in period 2011 - 2019

Character	Regression equation	DF	R^2	Significance
Viability of conserved seeds	35.073 – 3.732 U + 0.742V	9	0.91	$p < 0.001$

U = moisture of conserved seeds, V = initial viability

The regression coefficient R^2 shows that 91% of the variation of the conserved seeds' viability can be explained through the influence of these two factors. Of them, the moisture content has the largest influence on maintaining seed viability during conservation.

It has been found that the low moisture content of 6–7% provides very good conservation of the seed samples over a period of 8 years (2011–2018). Seed samples with a moisture content of 8–10% have recorded a maintaining viability percentage of approximately 65%, after 6 years of storage, and the ones with the moisture of 9–11% have recorded an average maintaining percentage of 60%, after 3 years of storage (2015–2018). At more than 16% moisture content, the viability percentage decreased on average by 17% (46–6%) even from the first year of conservation. The results do not highlight the influence of purity on the viability of the conserved seeds.

In the M2 environment, where the conservation temperature has been -3 °C, 11 seed samples have been stored, 4 from 2015 and 2016 and 7 from 2018. The results show a decrease of seeds' viability during the preservation period (2017–2019) on average by 34%, varying between 6% (Avrig 2018) and 86% (Avrig 2016). The highest viability losses have been recorded by the seed samples harvested from the Avrig seed orchard in 2016 and 2015 and Targoviste in 2015 and 2018. A positive significant correlation between the initial viability and the viability of seeds during conservation has also been found in this environment (Table 11).

Table 11. Correlations among qualitative parameters of seeds stored in the five conservation environments

Character	Seeds initial moisture	Seeds moisture in 2019	Seeds initial variability
Viability after 4 years in M1	-0.868**	-0.829*	0.666*
Viability after 3 years in M2	0.323	0.302	0.777**
Viability after 3 years in M3	0.200	0.203	0.813**
Viability after 3 years in M4	0.393	0.621*	0.571
Viability after 3 years in M5	0.433	0.398	0.672*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The better the viability of the seeds has been at the beginning of conservation, the higher it remained during the conservation period. No significant correlation between the conserved seeds' viability and their moisture content has been found.

In the M3 environment, where the conservation temperature has been -7°C, there has been a reduction of seed viability on average by 34% during the conservation period (2017–2019), ranging from 8% (Talisoara 2018) to 100% (Avrig 2016). The highest viability losses have been recorded by the seed samples harvested from the Avrig seed orchard in 2016 (100%) and Targoviste 2018 (56%). In this environment, it has also been found a positive significant correlation between the initial viability and the viability of the conserved seeds (Table 11).

In the M4 environment, with a conservation temperature of +5 °C, seeds' viability decreased on average by 55%, varying from 20% (Avrig 2018) to 100% (Avrig 2016). A positive, significant correlation between the seed viability assessed in 2019 and the seeds' moisture content has been found.

In the M5 environment (Seeds Conservation Centre at Brasov), where the conservation temperature has been +3 °C, the results show a reduction of seeds' viability on average by 51%, varying from 13% (Talisoara 2018) to 100% (Avrig 2015 and 2016). Significant correlations have been found only with the initial viability of the conserved seeds.

Taking into consideration the values of the qualitative parameters assessed in the five conservation environments, it can be concluded that the best conservation conditions have been the ones from M1 environment, at a conservation temperature of -5 °C (Fig. 3).

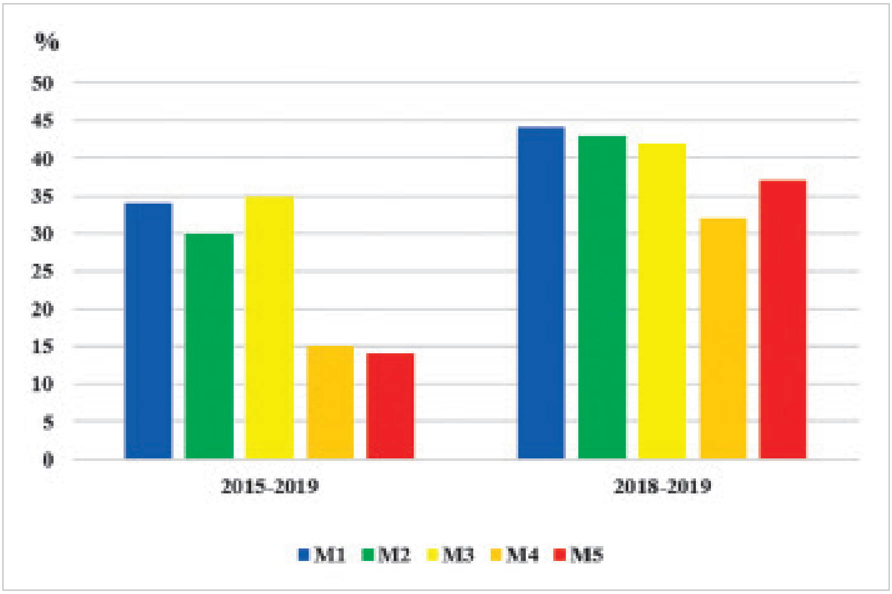

Fig. 3. Average percentage of viability maintenance for silver fir seed lots in the five conservation environments, during 2015 – 2019 and 2018 – 2019 periods

The seeds' conservation time depends on their viability at the beginning of storage but particularly on their moisture content. Drying silver fir seeds up to 6–7% moisture content, packing them in hermetically sealed polyethylene bags and storing them at -5 °C temperature (± 1 °C) allowed excellent conservation for 8 years. Drying seeds until a moisture content of 8–10%, packing and storing them in the same conditions led to good conservation for 6 years. The results have also demonstrated that for long-term conservation, the temperature of -7 °C is even better, provided that the seeds' moisture content is low.

Therefore, the seed moisture content and the temperature of conservation environment are the most important factors influencing the conservation of silver fir seeds. Similar results had been obtained by Barner (1978), who recommends a seed moisture content of 12–13% for conservation up to 3 years, but for a longer conservation time, the moisture content should be lowered to 7–9% at a storage temperature of -10 to -20 °C. Edwards (1974) further demonstrated that silver fir seeds could be preserved for 15 years at a moisture content of 5–8% and conservation temperature of -10 to -17 °C. Results obtained for other species also support the fact that the moisture content of the seeds and the storage temperature are the most important factors upon which the conservation of forest species seeds depends, but the values of these parameters are specific for each species (Roberts 1961, Suszka 1974, Hong et al. 1996, Bonner & Karrfalt 2008, Budeanu 2018).

In addition to the initial qualitative parameters of the seed lots, the manner in which the cones and seeds have been processed significantly influenced their viability during conservation. Thus, the highest viability losses have been recorded in the case of seed lots from the Targoviste seed orchard because the seeds have been processed by wings removal. This technique, not allowed by national and international standards, led to the breaking of the resin pockets and caused damage to the seed tegument, which subsequently led to rapid loss of the embryos' viability because in the case of this species the wing intergrows with the seed.

The fructification year represents another important factor. The poorest results have been obtained in the case of the seed lot harvested in 2016, which has been a year with poor fructification for silver fir in Romania. Therefore, the quality of silver fir seeds correlates strongly with the fructification year and the basic material from which the seed lots comes. The quality of seeds harvested from seed orchards, in years with good fructification, is superior to the one from seed stands or a year with weak fructification.

The packaging type has also been found to influence seeds' viability during conservation. All seed lots preserved in the five cold rooms have been packaged in polyethylene bags. In the case of the Avrig 2015 seed lot, stored in the M1 environment, the seeds had been packaged only in raffia bags. This type of package allowed moisture exchange with the storage environment, which explains the extremely low viability percentage obtained by this seed lot (28%) compared with the other seed lots harvested in the same year.

4.2. Assessment of silver fir seeds' respiration in conservation environments

Seeds of the forest species are living organisms, and because of this, the physiological processes that occur at metabolic and structural levels in seeds continue during the conservation period. The rate of seed's respiration is the most important physiological parameter in the case of seed conservation because it is closely related to metabolic activity. Through respiration, seeds consume the nutrients stored in the endosperm as well as oxygen, generating carbon dioxide, water and energy in the form of heat.

Even under optimum storage conditions, seed viability decreases with storage time. However, this process is more rapid at higher seed moisture, higher storage temperature and higher oxygen level in the seed lot (Barton 1961, Harrington & Jonnson 1970). To ensure optimum conservation conditions, the seed's respiration should be reduced to a minimum but not completely stopped, so that the embryo's viability wouldn't be damaged.

Seed respiration rate depends on the species, seed's development/ maturation stage, moisture content and storage temperature (Sorour & Uchino 2004, Chidananda et al. 2014). Fresh, completely developed and mature seeds have high metabolic activity, and therefore a higher rate of respiration. Species with higher natural moisture content (common beech, oaks, horse chestnut tree) also have a higher respiration rate. The rate of respiration decreases in the case of damaged or very old seeds due to low metabolic activity (Bewley & Black 1983).

The respiration rate has been assessed for 11 seeds samples belonging to the fructification years 2015, 2016 and 2018, which have been conserved in the 5 refrigerating chambers for 3 years and one year, respectively. Seed respiration rates varied significantly depending on the conservation temperature but also on the year of fructification. Furthermore, there is a genetic variation determined by the seed lot (Table 12).

The regression equation and the main factors influencing silver fir seed's respiration during conservation are given in Table 13. Thus, the most important factors explaining 81% of the variation in this characteristic are the temperature of the conservation environment, moisture and initial viability of the seeds. Silver fir seed's respiration rate has been correlated positively and highly significant with these factors.

Table 12. Analysis of variance for respiration rate of conserved silver fir seeds

Source of variation	DF	Variance (s^2)	F_{exp}	Significance
Seed lot	10	20.959	30.583	$p < 0.000$
Conservation environment	4	2.381	3.474	$p < 0.050$
Year of fructification	2	32.773	47.843	$p < 0.000$
Error	54	0.685		

Table 13. Influence factors and regression equation for respiration rate of conserved silver fir seeds

Character	Regression equation	DF	R^2	Signification
Respiration rate of conserved seeds	$-4.879 + 0.117T + 0.379 U + 0.034V$	3	0.81	$p < 0.000$

The highest respiration rate values have been recorded for seed samples harvested in 2018 (Fig. 4, 5). In the first place, there has been situated the Gârcina 2018 seed sample, in all deposits, with respiration rate values between 4.664–8.433 ugCO_2/g/seed/minute, because the seeds' moisture at the conservation time had the highest values (25%). The other seed samples harvested in 2018 followed, with the moisture content between 15 and 17%.

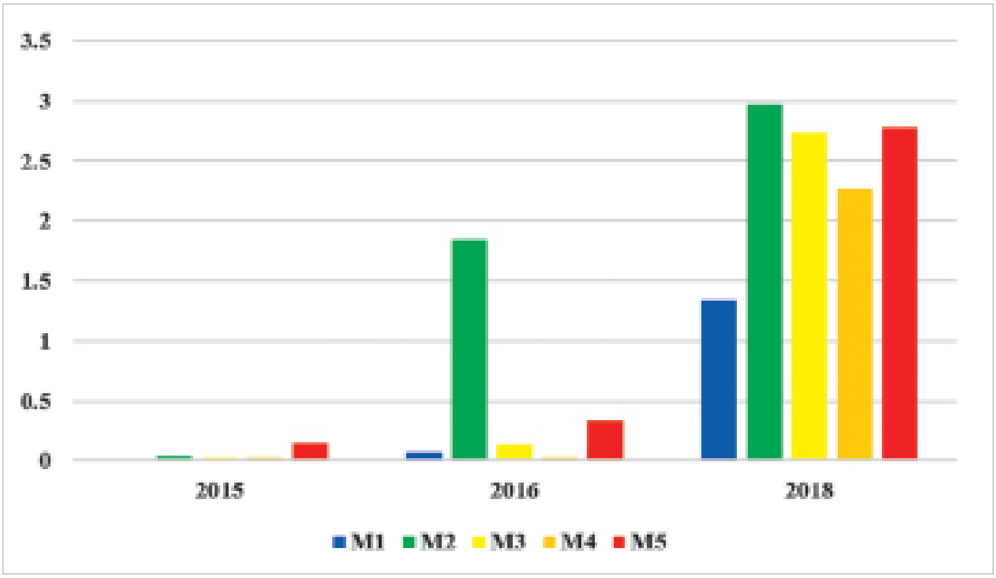

Fig. 4. Respiration rate of silver fir seeds from fructification year 2015, 2016 and 2018 in the five conservation environments

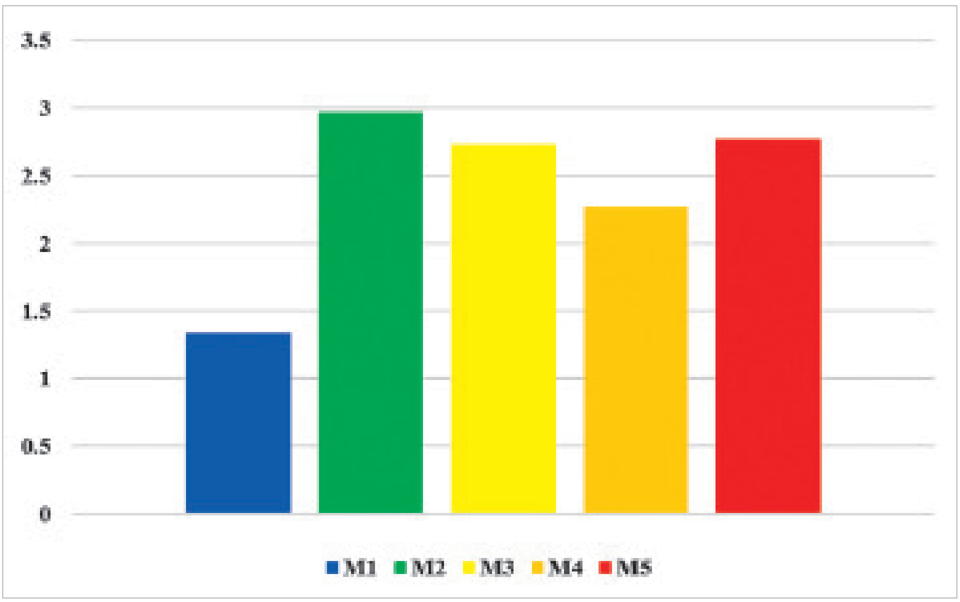

Fig. 5. Respiration rate of silver fir seeds from fructification year 2018, in the five conservation environments

The lowest respiration rate has been recorded for seed samples from 2015 (0.004–0.090 ugCO_2/g/seed/minute) with a moisture content of 8–12% (Fig. 6).

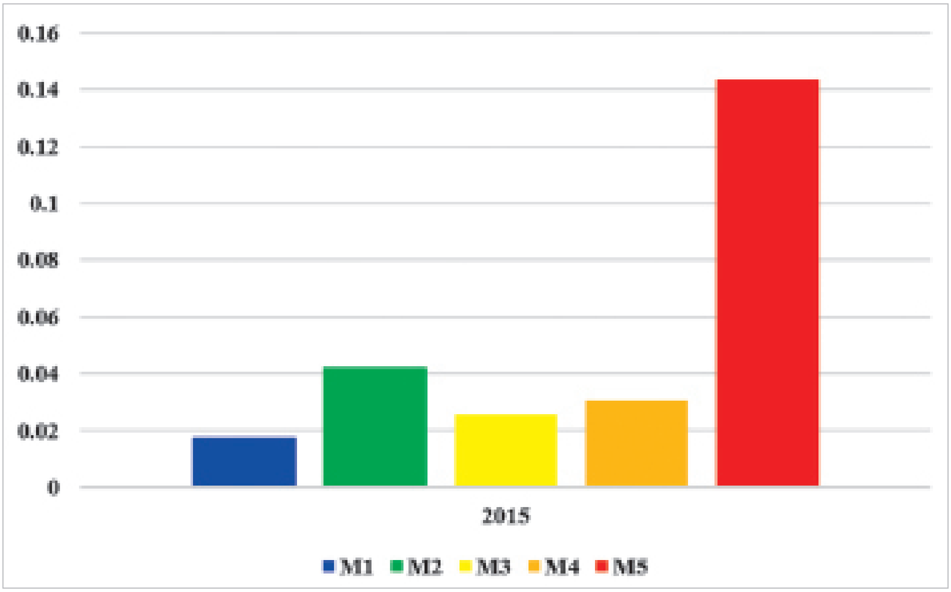

Fig. 6. Respiration rate of silver fir seeds from fructification year 2015, in the five conservation environments

Analysis of results showed that the lowest respiration rate has been recorded in the M1 environment (-5 °C), followed by M3 (-7 °C), and the most active respiration has been founded in M5 (+3 °C). Intermediate values (somewhat lower) for seeds respiration rate recorded in M4 (+5 °C) can be explained by the fact that all seed samples in this storage recorded the highest percentages of dead seeds. High temperature in this environment, most likely, led to a higher initial respiration rate, leading to a decrease in seed's viability during the conservation period.

One of the respiration's effects during storage has been the change of the initial moisture content of the seeds by +3% to -3% on average. All samples recorded a decrease in moisture content, on average by -3%, in the M4 environment at the temperature of +5 °C. Furthermore, the seed samples collected in 2015 registered an average moisture content increase of +3% in the M5 environment at a temperature of +3 °C and for seed samples from 2018, the seeds' moisture content has been kept constant only in M2 (-3 °C). From the results presented, it has been found that the most stable conservation environment is M1 (-5 °C) for the analysed period (3 years) because it determines the lowest fluctuation of the qualitative and physiological parameters of the seeds during storage. Wang (1974) and Stein et al. (1974) noted that repeated fluctuations in seeds' moisture content led to a loss of seed viability and therefore, as far as possible, the storage temperature should be constant.

Polyethylene bags have been found to be, from this point of view, a suitable packaging type because they are impermeable in terms of moisture exchange with the environment but allow a slight exchange of oxygen and carbon dioxide (Pasquini et al., 2012). In our experiment, polyethylene bags have been sealed in such a way that the air quantity was as low as possible inside the seeds' mass. The reduction of oxygen concentration in the lot's mass is also highly important for the long-term conservation of seeds. Hong et al. (2005) and Groot et al. (2015) showed that the ageing of seeds with low moisture content is accelerated by the presence of oxygen in the storage environment. Therefore, they recommended for seeds to be preserved under anoxic conditions to prolong longevity during *ex situ* conservation.

4.3. Pre-treatments to stimulate germination capacity of conserved silver fir seeds

The silver fir is one of the species whose seeds exhibit a slight physiological dormancy (Edwards 1974). This is demonstrated by the fact that, even when environmental conditions are ideal for growth, the fresh collected seeds remain inactive and germinate just after a long period (between 21 to 28 days under laboratory conditions, SR 1908/2004). Also, conserved seeds with low moisture content, show this tendency to germinate with difficulty. In their case, the carbon dioxide resulting from the respiration process is accumulated in the seed lot's mass and acts as an inhibitor on the embryos, causing a secondary dormancy.

The seedlings quality decreases with the increase of the emergence period in the nursery, thus emphasizing the importance of rapid germination. To remove the dormancy and shorten the germination period, the silver fir seeds have been subject to two treatments: moisturising and cold storing (T1) and moisturising before sowing (T2). Untreated samples, as control samples, constituted the third treatment (T3).

The first treatment involved placing the conserved seed samples in plastic boxes with a lid (25 cm x 15 cm x 3 cm) on a substrate of filter paper which was moistened with 50 ml water. The boxes have been placed in polyethylene bags which have been sealed and then stored in the refrigerator at 4 °C for 25 days. The second treatment consisted of wetting the seeds in water for 10 minutes before sowing. There have been tested 12 seed samples from different fructification years: 2 samples from 2010, 3 samples from 2013, 3 samples from 2015 and 4 samples from 2018. The analysed samples had been formed on average of 200 randomly selected seeds from each conserved lot. The seed samples of the fructification year 2018 have been subject to T2 treatment only.

After the cold storage period according to T1 procedure, the seeds have been sown in the nursery field on the 17th of May 2019 together with the seeds treated according to T2 and with the untreated seeds (T3). Assessments in the nursery have been conducted periodically at an interval of seven days, until 30 July 2019.

Results show that all samples treated under T1 procedure manifested the highest germination energy (Fig. 7). Although these seeds had a duration of conservation of 8, 5 and 3 years, respectively, they emerged the first in the nursery after 2 weeks, and the maximum percentage of germination was recorded during the next two weeks (in the middle of June). Differences between the T1 treatment and the other two treatments concerning germination energy are highly significant ($p < 0.001$) (Table 14).

Table 14. ANOVA for germination energy, emergence percentage and seedlings height in nursery, in relation to the pre-treatments applied to seeds conserved for 4 years in M1 environment (-5°C)

Source of variation	Variance (s^2)		
	Germination energy	Emergence percentage	Height of seedlings
Treatments	879.723***	11.069	16.555***
T1 vs T3	1036.012*	45.762	26.670***
T1 vs T2	1057.190*	14.583	20.526***
T2 vs T3	20.417	256.267	0.710
Error	103.083	380.181	0.948

Treatments: T1= moistening conserved seeds and storing them at temperature 4ºC for 28 days before sowing, T2 = moistening conserved seeds for 10 minutes before sowing, T3 = seeds conserved and untreated. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

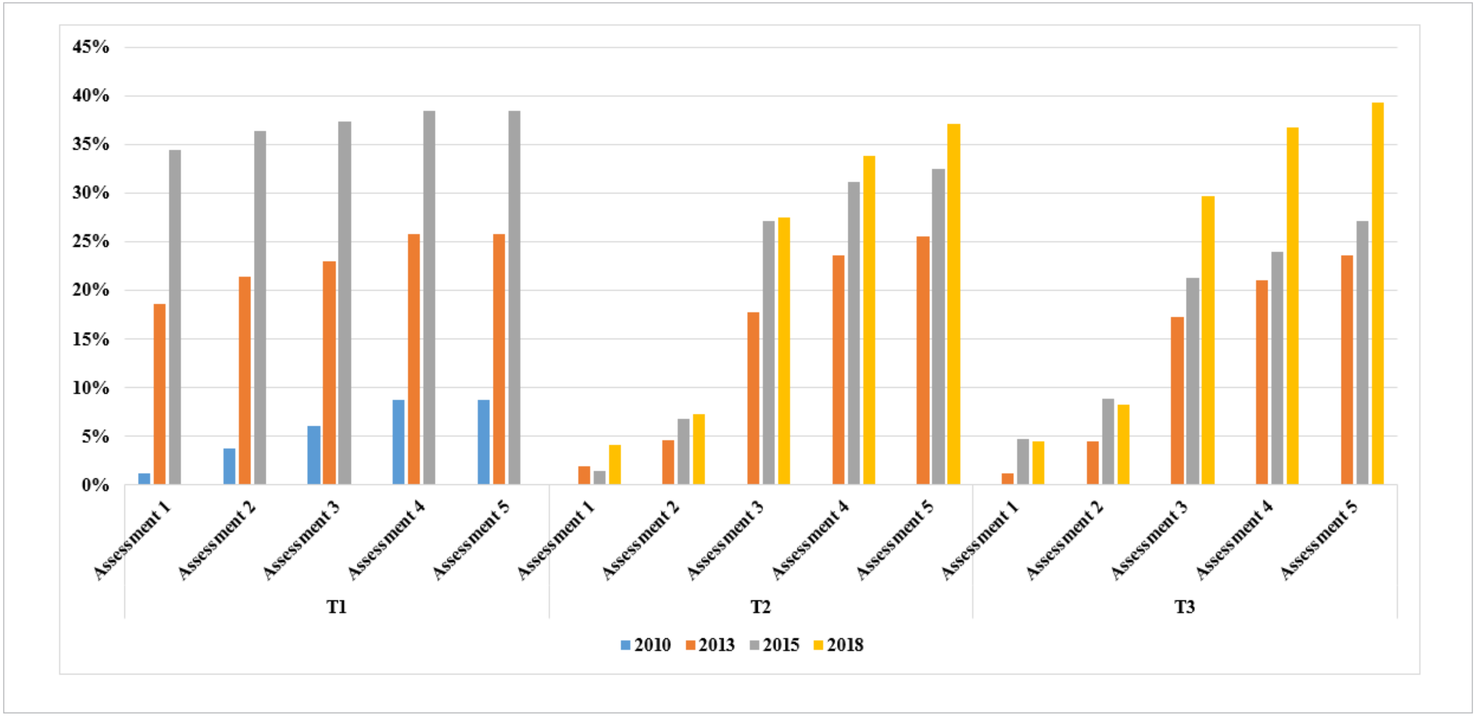

Fig. 7. Emergence percentage of silver fir seed samples conserved in M1 environment (Sibiu), after applying treatments to stimulate germination capacity. T1, T2 and T3 are the applied treatments, 2010 - 2018 are the harvest years.

No significant differences have been recorded between T2 and T3 treatments (Fig. 8). All seeds subject to T2 treatment and the control samples (T3) started to spring 4 weeks after sowing (10 to 18 June), and the maximum percentage has been recorded after another 4 weeks, ending their emergence in the middle of July.

There have also been noted differences regarding the germination/emergence capacity in the nursery depending on the seed orchard (Fig. 8). Regardless of the treatment and the harvest year, the highest germination energy has been recorded in the seed lots harvested in Tălișoara (Șugaș, Covasna) seed orchard.

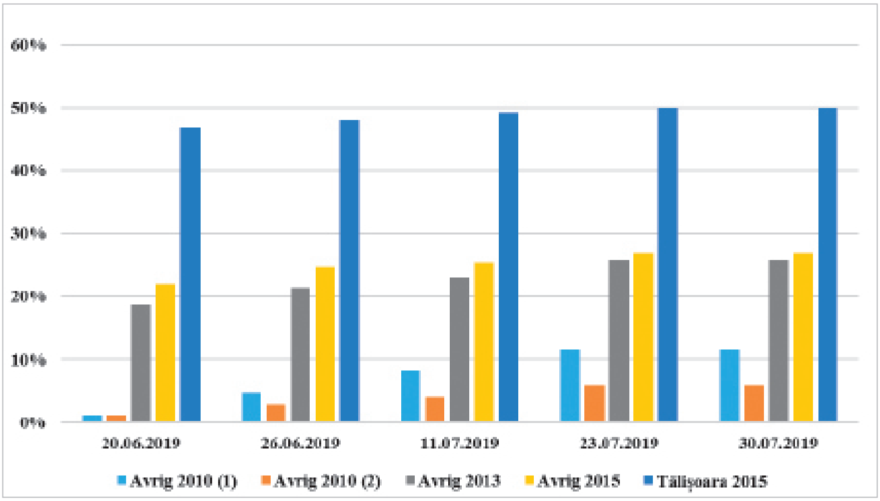

Fig. 8A Emergence percentage of silver fir seed lots conserved in M1 environment, after applying treatment T1

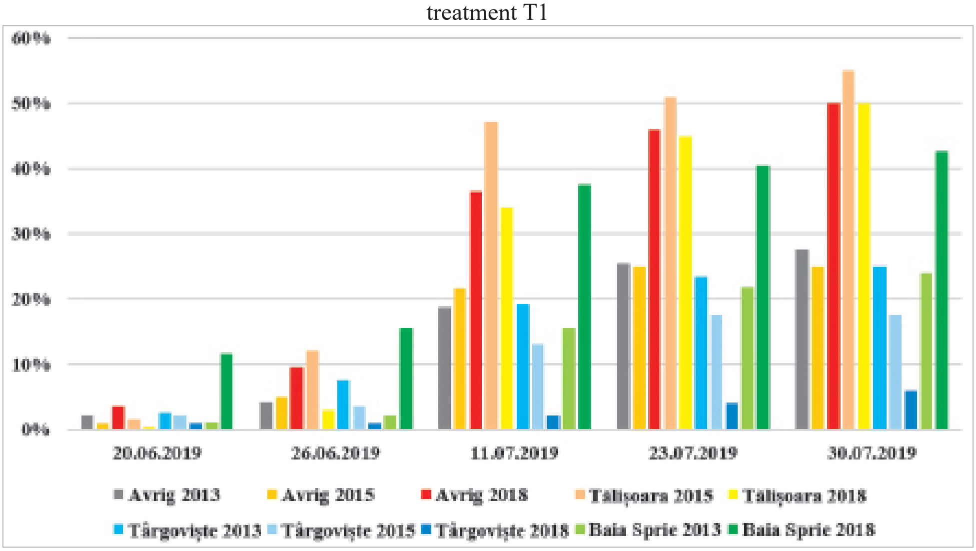

Fig. 8B Emergence percentage of silver fir seed lots conserved in M1 environment, after applying treatment T2

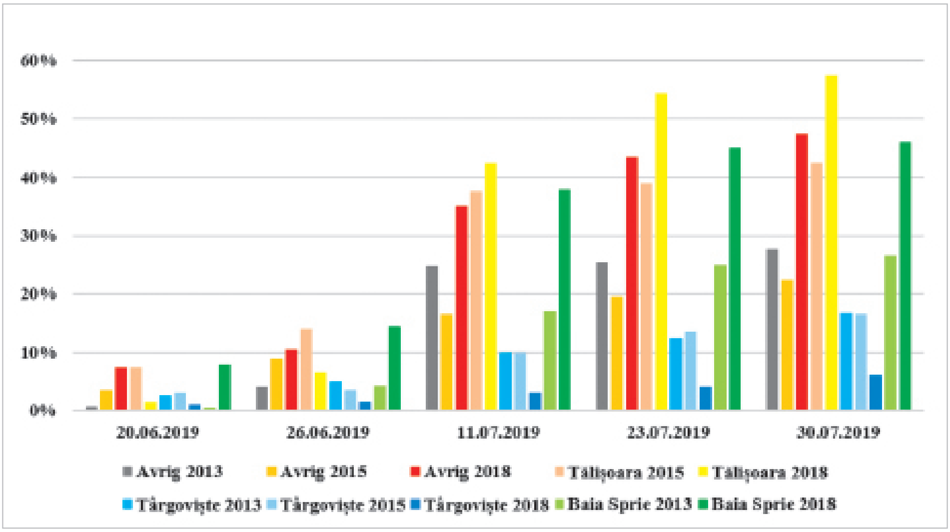

Fig. 8C Emergence percentage of silver fir seed lots conserved in M1 environment, after applying treatment T3

4.4. Characteristics of seedlings grown from conserved silver fir seeds

At the end of the 2019 growing season, the height increment of the seedlings obtained from the seeds subject to the treatments presented in the previous chapter has been evaluated. The seedlings obtained from seeds treated according to T1 procedure have been higher (3.22 cm) compared to the seedlings obtained through the other two treatments (2.70 cm and 2.62 cm, respectively).

The differences between T1 and T3 treatments, and T1 and T2, in terms of the height increment of seedlings, have been highly significant (Table 14). The performances recorded by the seedlings differed, also, significantly depending on the seed orchard. Therefore, the highest increase has been recorded in the seedlings emergent of seeds coming from the Tălișoara seed orchard (3.22 cm) and the smallest by the ones from the Baia Sprie seed orchard (2.35 cm).

5. RECOMMENDATIONS REGARDING HARVESTING AND PROCESSING OF SILVER FIR CONES AND SEEDS

In this chapter, we will discuss the main problems related to the harvesting and processing of the silver fir cones and seeds, which can negatively influence the seeds' quality and their conservation, as well as the success of the seedlings production in the nursery. Of these, we mention harvesting immature seeds, wrong storage techniques, and mistakes in cleaning, sorting and drying of cones and seeds.

One of the most important aspects is the moment of harvesting the silver fir cones. Cones harvesting should start when the seeds have reached physiological maturity. It is considered that silver fir seeds reach physiological maturity between the end of August until the beginning of October (Şofletea & Curtu 2001). For the same seed source, seed maturation varies from one year to another depending on the weather evolution in the respective period. In seed orchards, because trees' crowns are well lighted and they are located at low altitudes, which means warmer climate, silver fir seeds' maturity can take place faster, compared to seed stands, even in the second half of August. Also, in both seed orchards and seed stands, there has been a large inter-individual variation for this phenological character.

Between the maturation of silver fir seeds and the ripening of cones there is a quite close correlation. This allows the harvesting period to start when the cones become ripe until the scales open and the seeds disseminate. An important criterion for establishing the maturation of the seeds is the cones' colour, namely when the colour of the scales of the cones goes from green to brown and cones lose moisture and stiffness, and can be broken by hand. Another criterion for assessing seed maturity is the relative length of the embryo in relation to the cavity in which it is located. The recommendation is that the embryo should be at least 90% of the length of its cavity. It is important that seed harvesting is done when both the embryo and the endosperm are mature (the endosperm must have a solid and not milky consistency) because otherwise, the seeds lose their viability very quickly. Based on the analysis carried out we found that the degree of maturity of the harvested seeds influences both the viability and the longevity of the conserved seeds.

In practice, for fear of losing fructification, cones harvesting is usually made when they are not yet fully ripped. Some studies show that seeds from still green cones, which have not reached their full ripeness, can have a higher germination capacity than seeds from completely ripe cones (Vlase et al. 1957). This indicates that the maturity of silver fir seeds may precede cones ripening. For this reason, it is recommended in seed orchards for cones harvesting to be done individually, starting with the precocious clones, whose seeds reach physiological maturation faster. Therefore, a periodic analysis will be made regarding the seed development stage by harvesting and sectioning 1-2 cones of each clone from the seed orchard composition. For a more accurate analysis, cones can be sent to a forest seeds analysis laboratory.

Immediately after harvesting the silver fir cones, seeds are particularly susceptible to damage. During this period there is a great risk of losing seed viability. Because at the harvesting site, in the field, the climate fluctuations cannot be controlled, to avoid the depreciation of the seeds, the silver fir cones must be transported very quickly to the drying and processing warehouse. Until the transport is done the cones will be kept on tarps, under a cloth shelter. Keeping silver fir cones directly on the ground shall be avoided, as it increases the incidence of fungi. During transportation, the cones must be packed in paper or raffia bags, baskets or another type of package that permits good air circulation and seed respiration. The polyethylene bags, under no circumstances, shall be used for the transport of silver fir cones, because they lead to seed depreciation through overheating and mouldiness, especially when the transport is done over long distances.

The processing of silver fir cones will start with their drying in well-ventilated spaces, at a temperature that should not exceed 20°C. Cones storage at high temperatures and in poorly ventilated spaces will result in considerable losses in seed viability. Also, drying too fast at high temperatures can reduce viability. The surface of these spaces/rooms should be large enough so that the cones be stored in a single layer, in order to prevent the emergence of fungi. Throughout the drying period, the cones must be mixed daily to prevent moulding. Silver fir cones mould very easily due to their high moisture content. The mould penetrates through the seed's tegument and determines the death of the embryo.

After the seeds are separated from the scales and are sufficiently dried, they will be passed through sieves of different sizes to separate the scales and other impurities from the pure seeds. During the processing and sorting, the seed's wing removal will not be done and the separation of the scales will be done carefully, so as not to cause damage to the seed's tegument or to break the resin vesicles. Mechanical injuries caused by processing will cause rapid seed depreciation as they become a good medium for fungi development

(Harrington 1972). Breaking the resin vesicles and contaminating the embryo will lead to a decrease in the seed's viability. It has been found that healthy seeds, physiologically matured, with high initial viability, will maintain their viability longer during the conservation period. It is also important for the seeds to be well cleaned up of impurities and empty seeds, which are usually situated at the base and tip of the cones. It is recommended to use vertical air blowers and in no case the flotation method.

After sorting, the seeds are dried in a well-ventilated space, in a thin layer of a maximum of 3 cm, ensuring further periodical mixing. Samples will be taken periodically to determine the moisture content. It is important for seeds to dry evenly, to prevent moisture fluctuations in the mass of the seed lot during the conservation period, which would accelerate the respiration and transpiration of the seeds. When the moisture is suitable for preservation, the seeds will be packed in raffia bags and each raffia bag will then be placed in a polyethylene bag and will be sealed. The seed bags packed in this manner will be placed immediately in the refrigerating room at -5 °C.

6. CONCLUSIONS

6.1. Genetic variability of some morphological and qualitative characteristics of silver fir cones and seeds

The research outcomes highlighted a large intraspecific variability for the morphological and qualitative characteristics of the silver fir seeds and cones from the Romanian Carpathians. Genetic factor (provenance) influences the characteristics of silver fir seeds very significantly. Thus, the weight of 1000 seeds, has varied between 42 - 67g for studied populations, the most valuable populations from this point of view being: Dobra, Strâmbu Băiuţ, Anina and Sinaia.

Statistical differences have been highlighted according to the category of basic material also: selected sources vs. seed orchards. For the analysed characters, seed orchards obtained higher average values compared to populations. Thus, the weight of 1000 seeds, determined for seed orchards had values between 59 - 85g.

The variation amplitude of the characters has been higher for silver fir populations and the coefficients of variation have been lower for seed orchards, which indicates greater stability for the qualitative characters of the seeds obtained in seed orchards. Also, the variation at the level of open-pollinated families in selected trees from populations has been greater compared to the variation at the level of open-pollinated families of the plus trees in seed orchards.

The cones length and the weight of 1000 seeds have been correlated negatively with the altitude of the population's origin, indicating a clinal variation. No significant correlation has been found between the weight of 1000 seeds and the percentage of emergence in the nursery. However, there has been a significant positive correlation between the percentage of emergence and the growth of the seedlings in the first years in the nursery.

6.2. Conservation of silver fir seeds

The best conservation conditions for a period of 6 years have been at the storage temperature of -5 ^{0}C (± 1 ^{0}C) and a moisture content of the seeds between 8 - 11%, the loss of viability being 35%. It has been possible the conservation of the silver fir seeds for a period of 8 years at the same conservation temperature, but at a seed moisture content of 6-7%. The results also demonstrate that for long-term conservation, the temperature of -7 °C is even more indicated, on the condition that the seeds' moisture content to be low. In these conditions, the seed respiration rate had minimum values and the viability had maximum values.

The initial viability and moisture content of the seeds are the internal key factors for maintaining the seeds' quality during conservation.

The fructification year had a significant influence upon maintaining seed viability during the conservation period. The highest losses of viability in the conserved seeds have been recorded for the seed lot harvested in 2016, a year with poor fructification for silver fir in Romania.

In addition to the initial qualitative parameters of the seed lots, the processing of cones and seeds significantly influenced the percentage of viability during conservation. Processing silver fir seeds by removing wings must be avoided.

Polyethylene bags have been found to be a suitable packaging type because they are impermeable to the moisture exchange with the environment but allow a slight exchange of oxygen and carbon dioxide.

In order to remove the physiological dormancy and reduce the germination period of the conserved seeds, it is recommended to moisten them, placing them in polyethylene bags and keeping them at 4^{0}C for 25 days.

The results confirm the possibility of using the device „Insect Respiration Chamber" to assess the respiration of the seeds, as a method of monitoring the viability of the seeds during conservation. High-quality silver fir seeds production is largely dependent on adhering to the basic rules starting with harvesting and processing of cones, seeds extracting, processing and drying.

ANNEX

PROTOCOL FOR THE CONSERVATION OF SILVER FIR SEEDS HARVESTED IN SEED ORCHARDS

1. Flowering and development of cones and seeds

- Flowering: 1 - 20 April,
- Periodicity of fructification: 2 - 3 years,
- Evaluation of fructification: July 15 - August 1,
- Cones harvesting period: 20 August - 20 September. Periodic analyses must be done for the evaluation of physiological maturation of seeds,
- Average cones production per tree: 0.1 hl,
- Average seeds production per hectare: 300 - 400 kg.

2. Cones processing and seeds extraction

- Pre-drying of cones in well-ventilated spaces, at a maximum temperature of 20^0C,
- Single-layer placing for drying of the cones,
- Daily mixing of the cones, in order to prevent moulding,
- Sorting and cleaning the seeds of impurities carefully, without damaging them by wings removal or tegument scratching.

3. Qualitative parameters of silver fir seeds harvested in seed orchards

- Viability: 39 - 65%,
- Weight of 1000 seeds: 59 - 85 g,
- Number of viable seeds / kg: 6,600 - 10,000 pcs.

4. Silver fir seeds conservation

- Conservation environment temperature: -5ºC (±1ºC);
- Packaging type: polyethylene bags. The sealing of the bags must be done hermetically, so that the amount of air remaining inside is as low as possible,
- Seed lots' qualitative parameters required for conservation:
- seed viability must be high,
- the moisture content must be between 8 - 11% for a conservation period up to 6 years or 6 - 7% for a conservation period up to 8 years.

5. Factors affecting seeds production and conservation

- Fructification year: seeds harvesting will be done in the years with good or very good fructification,
- Climatic conditions during the flowering and cones development,
- Phenological differences between anthesis of male flowers and receptivity of female flowers,
- Cones harvesting moment: when the seeds have reached physiological maturity,
- Cones transport, storage and processing: in compliance with the basic rules,
- Seeds sorting, cleaning and drying: in compliance with the basic rules,
- Seed lots qualitative parameters: initial viability and moisture content,
- Conservation environment: temperature of : -5^0C ($\pm1^0$C);
- Packaging type: polyethylene bags are recommended, as they are impermeable in terms of humidity exchange, but allow a slight exchange of oxygen and carbon dioxide.

BIBLIOGRAPHY

BALDWIN, H.I., 1942. Forest Tree Seed. Chronica Botanica. Waltham, Massachusetts, 240p.

BALLIAN, D., 2013. Genetic overload of silver fir (*Abies alba* Mill.) from five populations from central Bosnia and Herzegovina. Folia Forestalia Polonica, series A, 55 (2), 49-57.

BARNER, H., 1975 B. The storage of tree seeds. In Report on FAO/DANIDA Training Course on Forest Seed Collection and Handling, No 2, FAO, Rome.

BARNER, H., 1978. Implementation of results from provenance research. Vol. 1, Proceedings of joint meeting of IUFRO Working Parties on Genetic Variation of Douglas Fir, Contorta Pine, Sitka Spruce and *Abies*, Vancouver, Brit. Columbia.

BARTON, L.V., 1961. Factors affecting longevity. Seed preservation and longevity. Interscience publishers, INC, New York. 30-46.

BEWLEY, J.D., BLACK, M., 1983. Physiology and Biochemistry of Seeds in Relation to Germination. Vol.2, Springer-Verlag, Berlin, 306p.

BONNER, F.T., KARRFALT, R.P., 2008. The Woody Plant Seed Manual. USDA Forest Service's, Agriculture Handbook 727, 85-95.

BOUVAREL, P., LEMOINE, M., 1958. Notes sur le reboisement: la conservation par le froid des graines de resineux. Revue Forestiere Francaise 10: 493–497.

BUDEANU, M., 2018. Conservarea semintelor cu longevitate naturala redusa la specii din familia Fagaceae. Editura Silvica, Bucuresti, 82p.

CERMAK, J., 1987. Monoterpene hydrocarbon contents of the resin from seeds of silver fir (*Abies alba* Mill.). Trees: Structure and Function, 1: 94–101.

CICEK, E., TILKI F., 2007. Seed Size Effects on Germination, Survival and Seedling Growth of *Castanea sativa* Mill., Journal of Biological Sciences, 7: 438-441.

CHIDANANDA, K.P., CHELLADURAI, V., JAYAS, D.S., ALAGUSUNDARAM, K., WHITE, N.D.G., FIELDS, P.G., 2014. Respiration of pulses stored under different storage conditions. Journal of Stored Products Research 59, 42-47.

DUNLAP, J.R., BARNETT, J.P., 1984. Influence of seed size on germination and early development of loblolly pine germinants. Can. J. For. Res., 13(1) : 40-44.

EDWARDS, D.G.W., 1974. The Woody Plant Seed Manual. *Abies fir P.* Mill. Victoria, BC, Canada: Forestry Canada, Pacific Forestry Centre, 149.

GAGOV, V., 1973. Izmenčivost pri semenata ot obliknovenata ela ot različni populacii v NR Bulgarija. Naučni trudovi na VLTI, Sofija, Tom 19, 51– 56.

GORIAN, F., PSQUINI, S., DAWS, M.I., 2007. Seed size and chilling affect germination of *Larix decidua* Mill. Seeds. Seed Sci. & Technol., 35, 508-513.

GOSLING, P., 2007. Raising trees and shrubs from seed. Practice guide. Forestry Commission: Edinburgh, 34p.

GROOT, S.P.C., DE GROOT, L., KODDE, J., VAN TREUREN, R., 2015. Prolonging the longevity of *ex situ* conserved seeds by storage under anoxia. Plant Genetic Resources: Characterization and Utilization, 13(1): 18–26.

HARALAMB, A., 1967. Cultura speciilor forestiere. Editura Agro-Silvică, Bucureşti, 778p.

HARRINGTON, J., JONNSON D., 1970. Maintaining seed viability in storage. Seed storage and packaging, The Rockefeller foundation New Delhi, 222p.

HARRINGTON, J.F., 1972A. Seed storage and Longevity. In Seed Biology III. Ed. by T.T. Kozlowski. Academic Press, New York, 145-240.

HOFFMANN, A.A., SGRO, C.M., 2011. Climate change and evolutionary adaptation. Nature 470: 479-485.

HOLMES, G.D., BUSZEWICZ G., 1958. The storage of seed of temperate forest tree species. Forestry Abstracts 19: 313-322, 455-476.

HONG, T.D., LININGTON, S., ELLIS, R.H., 1996. Seed Storage Behaviour: a Compendium. Handbooks for Genebanks: No. 4. International Plant Genetic Resources Institute, Rome, 98p.

HONG, T.D., ELLIS, R.H., ASTLEY, D., PINNEGAR, A.E., GROOT, S.P.C., KRAAK, H.L., 2005. Survival and vigour of ultra-dry seeds after ten years of hermetic storage. Seed Science and Technology 33: 449–460.

LAFFÉRS, A., 1979. Evaluation of seed weight in fir of Czechoslovakian and foreign provenance in relation to modified geographical latitude, to geographical longitude and to particular mountain ranges of Europe. Lesnícky časopis, 25, 111–125.

MIHAI, G., 2015. Îngrijirea, întreţinerea şi conducerea plantajelor gospodărite de RNP – Romsilva în vederea stimulării fructificaţiei. Manuscris INCDS Marin Drăcea, 35p.

MUHLE, O., STEPHEN, W., KLEINSCHMIT, J., 1985. Effect of seed size and weight on germination and growth of Douglus fir, Norway spruce and Scot pine. Forst und Holzwirt, 40(12) : 335-338.

NANSON, A., 2004. Genetique et amelioration des arbres forestieres [Genetics and tree breeding]. Les presses agronomique de Gembloux, Belgique, 712p.

PASQUINI, S., MIZZAU, M., PETRUSSA, E., BRAIDOT, E., PATUI, S., GORIAN, F., VIANELLO, A., 2012. Seed storage in polyethylene bags of a recalcitrant species (*Quercus ilex*): analysis of some bio-energetic and oxidative parameters. Acta physiologiae plantarum, 34(5), 1963-1974.

PARKER, W.H., NOLAND, T.L., MORNEAULT, A.E., 2006. The effects of seed mass on germination, seedling emergence and early seedling growth of eastern white pine (*Pinus strobus* L.). New Forests, 32, 33–49.

PÂRNUŢĂ, GH., LORENŢ, A., TUDOROIU, M., PETRILA, M., 2010. Regiunile de provenienţă pentru materialele de bază din care se obţin materiale forestiere de reproducere din România. Editura Silvică, Bucureşti, 122p.

PÂRNUŢĂ, GH., 2012. Catalogul Naţional al materialelor de bază pentru producerea materialelor forestiere de reproducere, Editura Silvică, Bucureşti, 300p.

ROBERTS, E.H., 1961. The viability of rice seed in relation to temperature, moisture content and gaseous environment. Ann. Botany 25, 381-390.

ROBERTS, E.H., 1973. Predicting the storage life of seeds. Seed Sci. Technol. 1, 499-514.

RUBŢOV, Ş., 1971. Ecologia şi cultura speciilor lemnoase în pepinieră. Editura Ceres, Bucureşti, 526p.

SABOR, J., 1984. Relation between the weight and the germination capacity of seed of silver fir. Sylwan, 4: 59-69.

SKRØPPA, T., TORLEIV T., 1990. Diallel crosses in *Picea abies*. I. Variation in seed yield and seed weight. Scandinavian Journal of Forest Research 5: 355-367.

SKRZYSZEWSKA, K., CHŁANDA, J., 2009. A study on the variation of morphological characteristics of silver fir (*Abies alba* Mill.) seeds and their internal structure determined by X-ray radiography in the Beskid Sądecki and Beskid Niski mountain ranges of the Carpathians (southern Poland). Journal of Forest Science, 55(9): 403–414.

SORENSEN, F.C., CAMPBELL, R.C., 1993. Seed weight - seedling size correlation in coastal Douglas-fir: genetic and environmental components. Can. J. For. Res. 23: 275-285.

SOROUR, H., UCHINO, T., 2004. The effect of storage condition on the respiration of soybean. Jpn. Soc. Agric. Mach. 66, 66-74.

STEIN, W.I., SLABAUGH, P.E., PLUMMER, A.P., 1974. Harvesting, processing and storage of fruits and seeds. In Seeds of Woody Plants in the United States, Agriculture Handbook No. 450. For. Service, USDA, Washington D.C., 98-125.

SUSZKA, B., 1971. Studies on the long-term storage of acorns. Annual Reports 1971-1974, Polish Academy of Sciences, Institute of Dendrology, Kornik, Poland.

SUSZKA, B., 1974. Storage of beech (*Fagus sylvatica* L.) seed for up to 5 winters. Arboretum Kornickie 19, 105-128.

ŞOFLETEA, N., CURTU, L., 2001. Dendrologie. Editura pentru viață, Brașov, 300p.

VLASE, I., DAMIAN, M., INAŞCU, M., 1957. Cîteva cercetări în legătură cu recoltarea timpurie a conurilor de brad. Revista Pădurilor, 1, 29-30.

WANG, B.S.P., 1974. Tree-seed storage. Can. Forestry Serv., Dep. Environ., Ottawa. Publ. 1335, 32 p.

XU, Y., CAI, N., HE, B., ZHANG, R., ZHAO, W., MAO, J., DUAN, A., LI, Y., WOESTE, K., 2016. Germination and early seedling growth of *Pinus densata* Mast. Provenances. J. For. Res. 27(2): 283–294.

***, Institutul Naţional de Statistică, 2018. www.insse.ro/

***, Institutului Forestier de Stat din Bavaria, 2019. www.lwf.bayern.de › biodiversitaet

***, Codul Silvic. Legea 46/2008.

***, International Rules for Seed Testing, Edition 2010, CH-8303, Bassersdorf, Switzerland.

***, International Rules for Seed Testing, Edition 2013, CH-8303, Bassersdorf, Switzerland.

***, International Rules for Seed Testing, Edition 2015, CH-8303, Bassersdorf, Switzerland.

***, SR 1908/2004, Seminţe de arbori şi arbuşti pentru culturi forestiere. Metode de analiză. ASRO, Bucureşti, 40p.

***, SR 1808/2004, Seminţe de arbori şi arbuşti pentru culturi forestiere. Eșantionarea loturilor de semințe. ASRO, Bucureşti, 40p

***, Recomandări tehnice. Pepiniere, metode şi procedee pentru cultura în pepinieră a principalelor specii forestiere şi ornamentale, Ministerul Apelor, Pădurilor şi Protecţiei Mediului, Bucureşti, 275p.

Seria a II-a **LUCRĂRI DE CERCETARE**

In the context of profound and fast climate changes, maintaining the stability and productivity of forests requires special efforts, as the natural processes reach to the limit. For maintaining and increasing adaptative capacity of the forest ecosystems, often natural regeneration must be supplemented or replaced by artificial regeneration works. This is either to increase the percentage of the resistant species to drought or high temperatures within the stands, or to reforest damaged or completely destroyed surfaces, by natural disasters.

European silver fir (*Abies alba* Mill.) is not only one of the main species of mountain ecosystems in Romania and Europe with multiple ecological and economic functions, but also a species important for the future, in a warmer and drier climate. In numerous provenance tests installed in many European countries, Romanian silver fir provenances have obtained superior growth and quality performances. Therefore, the interest for silver fir seeds from Romania, especially from seed orchards, is continuously increasing not only in the country but also for export.

Based on a diverse research material and by applying appropriate scientific and statistical methods, for the first time in Romania, it has been possible to elaborate concrete and detailed recommendations for the long-term harvesting, processing and conservation of silver fir seeds, without major losses in germination capacity. The book includes various stages regarding the process of silver fir forest reproductive material production, starting with cones and seeds harvesting and processing, packaging and conservating them at optimum temperatures, pre-treatments necessary to stimulate the germination capacity before sowing and the seedlings characteristics in nursery depending on the seed's quality. Significant differences have been found in the genetic variability of the morphological and qualitative characters of the cones and seeds, the superiority of the seed from orchards seeds, as well as the new method for the respiration rate assessment of seeds during conservation adapted from the entomology field, have a great scientific importance. Also, the results are particularly important for the forestry practice, both for the forest districts, which harvest and process cones and seeds, for nurseries, that produce and provide the seedlings needed for reforestation, as well as for forestry specialists in production, planning and research. By compliance to the "Protocol for the conservation of silver fir seeds" presented in the book's final chapter, providing continuous base of high-quality silver fir seeds will be possible.

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