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Improving seed germination and seedling emergence in the *Juniperus communis*

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Abstract: The observed juniper decline, lack of natural regeneration, and disappearance of numerous populations of the common juniper in Poland, were the major reasons for initiation of research on its sexual propagation and seedling production. This study shows that seed dormancy in this species is broken by warm-cold stratification at 15°C/3°C, for 14+12 weeks, respectively. Seed drying for 72 h at room temperature in the middle of the warm phase (i.e. after 4–8 weeks of stratification at 15°C) and the following cold stratification at 3°C, cause a significant increase in seed germination capacity at cyclically alternating temperatures of 3~15°C, and in seedling emergence in growing trays (67 cells each) in a greenhouse. However, seedling emergence was over 2-fold to 4-fold lower in an open nursery than in the greenhouse. After sowing in the nursery, secondary dormancy was probably induced, because some seeds germinated in the following year. Some very young, 2-year-old seedlings started to produce male or rarely female cones. This study also showed that soil conditions of mother plants can influence the pattern of seed germination and seedling emergence.

Additional key words: common juniper, berry-like, seed cone, propagation, individual variability

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Introduction

The natural range of the common juniper covers nearly the whole Northern Hemisphere, extending from North America to Europe, North Africa, Siberia, China and Japan. Among woody plants, this species has the largest natural range. However, in the second half of the 20th century, a dramatic regression of the common juniper was observed in several countries of north-western Europe (Clifton et al. 1997, Broome 2003, Oostermeijer and de Knegt 2004, Verheyen et al. 2005, Ward 2003a, 2003b, Ward 2007, Ward and King 2006, UK BAP 2007). The lack of its natural regeneration seems to be due to an exceptionally high proportion of empty seeds or seeds damaged by insects (Verheyen et al. 2005), deep seed dormancy (Nikolaeva et al. 1985), and animal grazing (rabbits and deer). The most important factors affected seed yield include, e.g., a sufficient amount of pollen available to receptive ovules, which is conditioned to a large extent by the distance between parental shrubs, wind direction, and stage of ovule development (Mugnaini et al. 2007). Juniper seed cones from Polish populations can potentially contain 3 seeds, but some seed cones contain 4 seeds (Grześkowiak, oral comm., Garcia et al. 2000) or an even higher number of seeds, as Broome (2003) reported that in the British islands the mean number of juniper seeds is 4.2 per seed cone. These all above mentioned unfavourable factors, in combination with the increasingly noticeable climatic changes, i.e. long dry periods in spring and summer, decimate also the older junipers (Garcia et al. 2000, Tylkowski, data not shown).

The common juniper is a pioneer species, colonizing poor sites, e.g. inland dunes (Faliński 1980). In forests it performs an important biocenotic function, accelerating the decomposition of leaf litter, which has a positive effect on decreasing the soil acidity (Bobiński 1971, DeLuca and Zackrisson 2007). The berry-like seed cones are eaten by many birds, e.g. willow tits, marsh tits, crested tits, Bohemian waxwings, greenfinches, bullfinches, bramblings, blackbirds, robins, and others (Bobiński 1980). In the past, due to the ill-considered large-scale forest clearance, juniper bushes have completely disappeared from many parts of our country.

In spite of many signals about the decline or complete disappearance of some populations of the common juniper, as well as numerous appeals to foresters and naturalists for its protection in Poland, still too little attention is paid to this problem (Bobiński 1977, Boratyński 1985, Szczygielski 2007). Protection of single individuals of this species as 'nature monuments' will not stop the further disappearance of common junipers from the Polish landscape. Many local populations listed in the 1970s (Bobiński 1977) no longer exist or only their remnants have survived. Even in the Wolin National Park, in spite of the legal protection of the whole park, a fast rate of juniper decline is also observed (Tylkowski, data not shown). Leaving of old juniper specimens on cleared plots (aimed at self-sowing) also proved to be ineffective. Most of the seed cones are then affected by diseases, while seeds are eaten by insects, especially the juniper seed chalcid *Megastigmus kuntzei* (Kapuściński 1946, Bobiński 1976), which locally can destroy all seeds. This is one of the reasons why natural regeneration of this species is ineffective.

Under this serious threat, the conservation of the remaining juniper populations should be aided by means of sexual propagation in nurseries, followed by planting on suitable sites.

The aims of this study were: (1) to assess the seed yield from seed cones; and (2) to analyse the effect of seed drying during stratification on seed germination in laboratory conditions, and on seedling emergence in the greenhouse and in the nursery.

Materials and methods

For the assessment of seed yield from seed cones, juniper seed cones were collected in early September 2003 in the Rytel Forest District (N 53°44'50'', E 17°49'45'') from 7 shrubs growing under a pine canopy on a potential site of fresh mixed coniferous forest, and in 2006 from 12 shrubs in the Rajgród Forest District and from 3 shrubs in the Waliły Forest District, also on potential sites of fresh mixed coniferous forest. From each shrub, all seed cones were collected by hand-rolling the twigs with cones. After cleaning, the seed cones were counted and weighed; next, they were crushed and the seeds were removed. The seeds were separated from remnants of the pulp by rolling them in bags with fine sand. The seeds were then soaked in water, to separate the good (filled) seeds from empty seeds, which floated on the water surface. After drying of the filled seeds for a week at room temperature, they were counted and weighed, and on the basis of those measurements some additional parameters were calculated, i.e. mean seed cone weight (for each shrub), seed yield per seed cone, and percentage contribution of filled seeds to the total weight of seed cones.

For the analysis of the effect of seed pre-treatment before sowing (i.e. seed desiccation during stratification) on seed germination and seedling emergence after these procedures, seeds were collected from 3 shrubs in October 2005. Shrubs A and B grew on potential sites of fresh coniferous forest in the Wieluń Forest District (5980 and 4124 filled seeds, respectively), while shrub C on a potential site of dry coniferous forest, i.e. on an inland dune in the Grodziec Forest District (3640 filled seeds). Only the seed lots with very numerous filled seeds were selected, because a relatively large number of seeds was necessary for the experiments. In the case of shrub B, one experimental variant was deliberately omitted (sowing in the nursery) because of an insufficient number of seeds.

After seed extraction from the cones and separation of filled seeds from empty seeds, the filled seeds were dried at room temperature to a moisture content of ca. 10%, and next were stratified in a moist sand-peat substrate (1:1, v/v, fine quartz sand and de-acidified sphagnum peat with pH 5.5–6.5), in the warm-cold system at 15°C/3°C. The warm phase lasted 14 weeks and the cold phase lasted 12 weeks, i.e. till the beginning of seed germination.

The warm phase of stratification was interrupted once for 3 days (72 h), i.e. after 2, 4, 6, 8, 10, 12 or 14 weeks (corresponding to experimental variants: *b*, *c*, *d*, *e*, *f*, *g*, *h*). Variant *a* was the control, where no drying was applied during stratification. Seeds were then extracted from the stratification substrate, and dried on blotting paper at room temperature. After the 3 days, stratification was continued according to the above-mentioned experimental design.

After stratification, the seeds (in 3 replications of 50 seeds each) were subjected to:

- a germination test at cyclically alternating temperatures of 3~15°C (in 24-h cycles for 16 h at 3°C and next for 8 h at 15°C), in the sand-peat substrate described above;
- an emergence test in a greenhouse (more precisely, a plastic tunnel): sowing in a peat-perlite substrate in plastic growing trays HIKO V-50 (BCC, Landskrona, Sweden);

- an emergence test in a nursery: sowing in the forest soil on 28 March 2006.

In the greenhouse, each growing tray was composed of 67 cells (each 50 cm^3 in volume), filled with a mixture of sphagnum peat (pH 5.5–6.5) with perlite (Agra-perlite No. 3, with grain fraction up to 6 mm), at a ratio of 2:1 (v/v), with addition (per 1 m^3 of substrate) of 1.5 kg of Osmocote® Classic Standard fertilizer (NPK fertilizer containing magnesium with trace elements 15+9+9 (+3.0), longevity 5–6 months).

In the nursery, the seeds were sown in furrows impressed in the podzolic soil, to the depth of 1 cm. The seeds were next covered with a 1-cm layer of sand and a 3-cm layer of milled pine bark.

Both in the greenhouse and in the forest nursery, the substrate was regularly moistened with water from the nearby Lake Kórnickie.

During germination tests, a seed with a radicle at least 1/3 as long as the seed was considered as germinated. On the basis of weekly observations, a germination curve was plotted and their germination capacity was estimated.

In the first growing season, seedlings were counted in the greenhouse and in the nursery. In mid-August, the plastic cover was removed, to enable hardening before winter. The trays in the greenhouse and the patches in the nursery were left undisturbed for the winter, and in spring 2007 some seedlings germinated from the seeds that remained ungerminated in the preceding year. Also after the growing season in 2007, the patches and trays were left undisturbed to observe emergence in 2008.

In 2006, data on seed germination and seedling emergence in the greenhouse were transformed according to the function *arc sine* $\sqrt{\%}$, and next subjected to analysis of variance, by using Statistica software (1998), and to Tukey test at P = 0.05.

Results

Seed yield

From individual shrubs, from 58 to 3040 seed cones were collected (Table 1). Mean seed cone weight was 0.123 g, and varied from 0.083 g for shrub 6, to 0.189 g for shrub 1. This shows that the mean value for shrub 6 was nearly 2.4-fold lower than for shrub 1.

Table 1. Mean values of the studied juniper seed yield parameters for individual shrubs from three forest districts

Shrub		Number of seed cones	Total weight of seed cones (g)	Seed cone weight (3/2) (g)	Filled seeds		Weight of 1000 filled seeds (g)	Contribution of filled seeds to seed cone weight (5/3) (%)	Number of filled seeds per cone (6/2)
Provenance	No.				Weight after drying (g)	Number			
1		2	3	4	5	6	7	8	9
Rytel	1	939	186.1	0.198	30.536	1777	17.13	16.41	1.89
	2	1921	174.2	0.091	24.314	2765	8.78	13.96	1.44
	3	1481	176.0	0.119	11.181	1010	11.18	6.35	0.68
	4	1828	219.0	0.120	26.902	2695	10.00	12.28	1.47
	5	2186	239.0	0.109	33.805	2620	13.16	14.14	1.20
	6	443	36.9	0.083	5.342	367	14.56	14.47	0.83
	7	300	43.6	0.145	3.650	275	13.27	8.37	0.91
Rajgród	8	551	63.318	0.115	9.091	730	12.45	14.35	1.32
	9	795	120.97	0.152	14.441	1415	10.21	11.94	1.78
	10	58	7.946	0.137	0.668	35	19.08	8.41	0.60
	11	735	76.836	0.105	0.916	73	12.55	1.19	0.10
	12	1425	129.65	0.091	1.265	100	12.65	0.97	0.07
	13	2845	366.45	0.129	48.933	3575	13.69	13.35	1.26
	14	363	34.88	0.096	1.232	93	13.25	3.53	0.26
	15	285	29.60	0.104	3.608	315	11.45	12.19	1.10
	16	1333	219.60	0.165	6.054	605	10.01	2.76	0.45
	17	259	32.77	0.126	4.175	400	10.44	12.74	1.54
	18	529	65.21	0.123	6.324	495	12.77	9.70	0.94
	19	589	68.12	0.116	13.400	980	13.67	19.67	1.66
Walily	20	765	119.93	0.157	7.656	445	17.20	6.38	0.58
	21	1000	116.16	0.116	8.340	605	13.78	7.18	0.60
	22	3040	312.21	0.103	28.746	2520	11.41	9.21	0.83
		Mean		0.123			12.85	9.98	0.98

The potential number of filled seeds per seed cone should reach 3, but in fact it was lower and ranged from 0.07 (for shrub 12) to 1.89 (for shrub 1). This is due to the fact that many seed cones may contain no filled seeds, while others may contain 1–3 seeds. The percentage contribution of filled seeds to the total weight of seed cones varied from 0.97% (shrub 12) to 16.41% (shrub 1), while the man value for all 22 shrubs was 9.98%. Values of this parameter may be misleading and unreliable, because seeds from various shrubs may differ in seed coat thickness, whose contribution to the whole seed weight may be substantial, so the number of filled seeds per seed cone is a better measure of reproductive efficiency.

Weight of 1000 filled seeds was variable, and ranged from 8.78 g (for shrub 2) to 19.08 g (for shrub 10), on average 12.85 g. Thus a gram includes from 52.4 to 113.9 seeds, on average 77.8.

Effect of seed pre-treatment during stratification on seed germination and seedling emergence

In laboratory conditions at cyclically alternating temperatures of 3~15°C, germination capacity varied depending on seed pre-treatment (Fig. 1). Undried seeds (control) or seeds dried after 14 weeks of the warm phase of stratification, i.e. directly before the cold phase of stratification at 3°C, did not germinate at all or only a small percentage of them germinated during the germination test. The lowest proportion of seeds germinated for shrub C (5.3 and 0%, respectively) while about 30% seeds from shrubs A and B germinated in those variants. Values of germination capacity (Table 2) approximately match the parabola trend (Fig. 1), with the highest values of germination capacity when seeds were dried after 4–10 weeks of the warm phase of stratification. This rule is reflected in high correlation coefficients, from 0.6304 for seeds of shrub C, to 0.9039 and 0.9066 for seeds of shrubs A and B, respectively (Fig. 1).

Seeds of shrub A reached the highest germination capacity (76%) when dried between weeks 4 and 8, while seeds of shrub B (60–64.7%) when dried between weeks 4 and 10, and seeds of shrub C (57.3%) when dried after 6 weeks of the warm phase of stratification (Fig. 1). Germination capacity was significantly the highest when seeds were dried after 6 weeks of stratification, while the lowest germination capacity was recorded for seeds dried after 2, 4 or 8 weeks (these variants formed a homogeneous group).

In the first growing season (in 2006), in the greenhouse the level of seedling emergence (Table 2) was similar to germination capacity in laboratory conditions. The lowest proportion of seeds germinated for shrub C, not dried during stratification or dried after 14 weeks of the warm phase of stratification (2.7% and 0%, respectively) and seeds of shrubs A and B, whose germination capacity after the same pre-treatment reached 21.3–26.7%. The highest proportion of seeds germinated for shrub A, when they were dried after 4–10 weeks of stratification (81.3–88.7%), for shrub B also when dried after 4–10 weeks (56.0–63.3%) and for shrub C when dried after 2–6 weeks (25.3–31.3%; Table 2). In respect of seedling emergence in trays (in contrast to germination capacity), a homogeneous group was formed by the variants where seeds were dried after 2–10 weeks of the warm phase of stratification (Table 2)

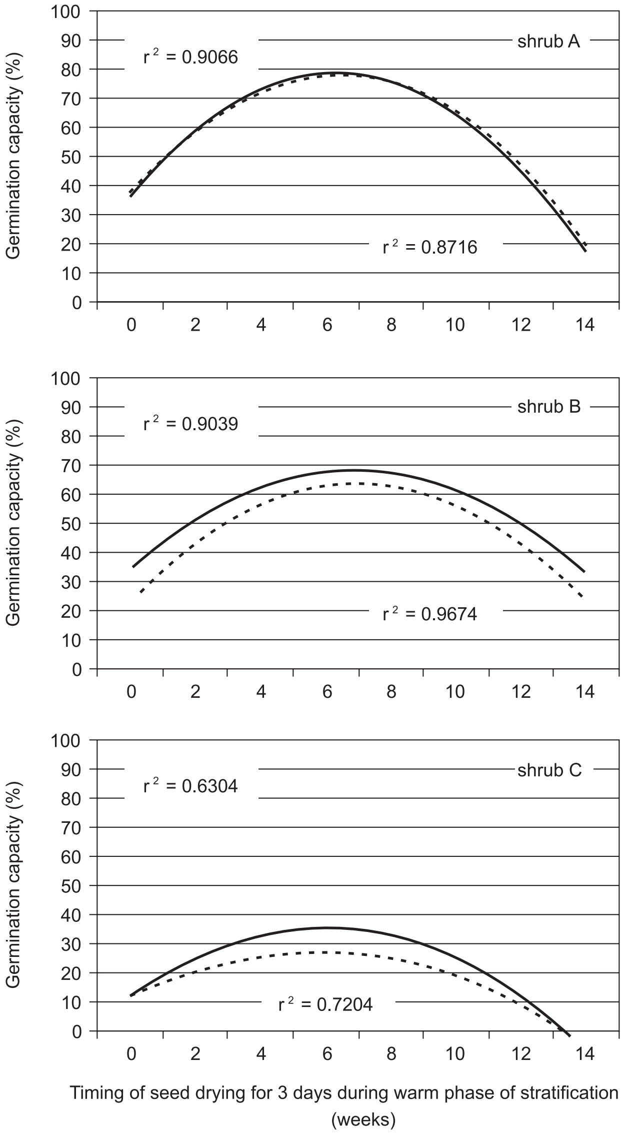

Fig. 1. Germination capacity trend line at 3~15°C (solid) and seedling emergence trend line (doted) and correlation coefficient in the greenhouse in 2006 of 3 seed lots

In general, the pattern of seedling emergence in the greenhouse was similar to germination capacity in laboratory conditions (Fig. 1), which is reflected in the trend line and similar values of correlation coefficient (0.7204 and for shrub C, 0.9674 for shrub B, and 0.8717 for shrub A).

Table 2. Effect of seed desiccation during stratification at 15°C/3°C on seedling emergence in trays in the greenhouse. Results from 2006 were tested with the Tukey test; the values marked with the same **letters** (**a**–**i** between seeding emergence, **A**–**D** between experimental variants and **X**–**Z** between skrubs) do not differ at $P = 0.05$

Experimental variant	Seedling emergence (%) in greenhouse								
	Shrub A			Shrub B			Shrub C		
	2006	2007	Σ	2006	2007	Σ	2006	2007	Σ
a (**D**)	26.7 **fg**	4.7	31.4	24.0 **fg**	24.7	48.7	2.7 **h**	28.0	30.7
b (**B**)	68.7 **ac**	4.0	72.7	42.0 **de**	10.0	52.0	31.3 **eg**	15.3	46.6
c (**A**)	80.0 **a**	6.0	86.0	56.0 **cd**	12.0	68.0	28.0 **eg**	5.3	33.3
d (**AB**)	75.3 **ab**	6.0	81.3	56.7 **cd**	11.0	67.7	25.3 **fg**	25.0	50.3
e (**AB**)	76.7 **ab**	12.0	88.7	63.3 **bc**	11.3	74.6	18.0 **g**	24.0	42.0
f (**B**)	60.7 **c**	21.3	82.0	60.7 **c**	18.0	78.6	19.3 **g**	23.3	42.6
g (**C**)	34.0 **ef**	26.0	60.0	42.0 **de**	39.3	81.3	3.3 **h**	26.0	29.3
h (**D**)	26.7 **fg**	30.0	56.7	21.3 **fg**	13.0	34.3	0.0 **i**	26.0	26.0
Mean	56.1 **X**	13.7	69.8	45.7 **Y**	17.4	63.1	16.0 **Z**	21.6	37.6

Table 3. Effect of seed desiccation during stratification at 15°C/3°C on seedling emergence in the nursery. ND = not determined

Experimental variant	Seedling emergence (%) in nursery									
	Shrub A			Shrub B			Shrub C			
	2006	2007	Σ	2006	2007	Σ	2006	2007	2008	Σ
a	4.7	12.7	**17.4**	1.3	22.7	**24.0**	0	38.7	0	**38.7**
b	16.0	7.3	**23.3**	ND	ND	ND	4.7	9.3	0	**14.0**
c	36.0	4.0	**40.0**	13.3	5.3	**18.6**	8.0	16.7	0	**24.7**
d	23.3	14.7	**38.0**	23.3	4.0	**27.3**	6.7	14.0	0	**20.7**
e	16.0	4.0	**20.0**	16.0	15.3	**31.3**	7.3	21.3	0	**28.6**
f	6.0	13.3	**19.3**	11.3	11.3	**22.6**	4.7	16.7	0	**21.4**
g	18.7	16.0	**34.7**	4.7	10.7	**15.4**	0.7	28.0	1.3	**30.0**
h	7.3	21.3	**28.6**	4.7	38.7	**43.4**	0.7	29.3	0	**30.0**
Mean	**16.0**	**11.7**	**27.7**	**10.6**	**15.4**	**26.0**	**4.1**	**21.7**	**0.2**	**26.0**

In contrast to the high seedling emergence recorded in trays in the greenhouse, poor emergence was observed in the nursery (Table 3). The lowest proportion of seeds germinated if they were not dried or were dried after 14 weeks of the warm phase of stratification, whereas the highest proportion when the seeds were dried between weeks 4 and 8. In general, however, seedling emergence in the nursery was over 2-fold to 4-fold lower than in the greenhouse, depending on mother plant and timing of seed desiccation. Unfortunately, in the case of shrub B, because of an insufficient number of seeds, variant *b* (= drying after 2 weeks of stratification) was deliberately omitted, but it can be presumed that by analogy to shrubs A and B, this result should be 40–50%.

Seedling emergence in trays in the first year differed significantly between mother plants (Table 2).

The mean height of 1-year-old seedlings in the greenhouse (ca. 10 cm) was on average 20% higher than in the nursery (data not shown).

It seems that the low seedling emergence in the nursery could be partly due to fungal pathogens present in the soil. In the greenhouse, where peat with perlite was used, no such threat occurred.

Seedling emergence in the second year

In spring, starting from 12 April 2007 both in the greenhouse and in the nursery, seedling emergence from seeds sown in the preceding year was observed (Table 2 and 3). Depending on mother plant, values of seedling emergence in the greenhouse reached from 5.3% to 30% of the initial number of sown seeds, and in the nursery from 4% to 38.7%. It should be noted, however, that the higher seedling emergence in the second year applied to the undried seeds and the seeds dried after the warm phase of stratification, i.e. the variants whose germination capacity in the preceding year was the lowest.

In the nursery, in the second year after sowing, mean seedling emergence from seeds of shrubs B and C was higher than in the first year, whereas in the greenhouse it was higher only for seeds of shrub C. Considering seedling emergence in both years, it can be noticed that only seeds of shrub A had a higher seedling emergence in the first year, both in the greenhouse and in the nursery.

The values of seedling emergence show that seeds strongly differed depending on mother plant. This may be due to the type of site occupied by the mother plants. The similar reaction of seeds from shrubs A and B to the applied desiccation, as well as the similar site type (fresh coniferous forest) remarkably differed from the reaction of seeds from shrub C, which grew on the poor and very dry potential site of dry coniferous forest (sandy inland dune).

Seedling emergence in the third year

In late April 2008, any seedling was recorded in the greenhouse (experiment was finished), whereas in the forest nursery, single seedlings emerged (only 1.3%) for seeds of shrub C, in the variant where seeds were dried after 12 weeks of the warm phase of stratification.

Seedling sex

In late April and early May 2008 (i.e. at the beginning of the third growing season), in the greenhouse ca. 20% of 2-year-old seedlings (40–50 cm high) started to produce male or female cones. Most of them were male and only few were female. The other seedlings have not produced any cones yet. In the forest nursery, single specimens also produced cones but fertilization probably did not occur because no seed cone growth and development was observed. Probably the high air temperatures in spring and summer in preceding year could have induced the formation of male and female cones in the very young plants.

Discussion

The number of seed cones per shrub depends on shrub age, health, and size (Bobiński 1976) as well as site altitude (Garcia et al. 2000). In some populations of the common juniper in Poland, every year there are large numbers of seed cones with visible holes made by insects feeding on seeds, or with empty seeds. Thus in such populations it is rather unlikely to collect a large number of viable seeds (e.g. many empty seeds were found in the forest districts Gidle and Szczytno). Thus before large-scale harvesting, it is worthwhile to check if the effort is sensible.

In the Rytel Forest District, seeds were collected from 7 shrubs with numerous seed cones, while several dozen other female shrubs had only small amounts, i.e. several dozen seed cones each. Similar seed cone yields were observed in 2006 in the forest districts Rajgród and Waliły.

It is hard to predict juniper seedling emergence in natural conditions (Broome, 2003). A warm spring may induce secondary dormancy in the seeds. Pack (1921) found that after stratification, dormancy was induced in juniper seeds when they were kept at >12°C. More predictable seedling emergence can be expected when stratified seeds are sown in controlled conditions. In this study, highly satisfactory germination capacity (from 57.3% to 76.7%) and seedling emergence (from 30% to 80%) in the common juniper was observed already in the first growing season after sowing (Table 2 and 3), but in the open nursery a large proportion of seeds started to germinate a year later. Broome (2003), after 60 weeks of stratification (seeds buried in the ground, exposed to natural weather conditions, and additionally to a low temperature, about 0°C, between weeks 30 and 50) followed by sowing in spring, reported seedling emergence of ca. 50–60% in the first growing season. She also found that seed stratification at 20°C for 13 weeks did not affect germination capacity, while freezing for a week at –13°C caused a significant decrease in germination capacity. Moreover, she suggests that soaking of common juniper seeds in 1% solution of citric acid or scarification in concentrated sulphuric acid, followed by cold stratification for 12 weeks, are sufficient for seed dormancy breaking. A similarly favourable effect of citric acid on seeds of *J. pinchotii*, *J. scopulorum* and *J. virginiana* was found by Johnsen and Alexander (1974) as well as Djavanshir and Fechner (1976).

Barton (1951) showed that seeds of *J. virginiana* after warm-cold stratification at 25°C/5°C, for 4 weeks and 3 months, respectively, had a high germination capacity (77%), and that the warm stage of stratification could be replaced by seed scarification for 30 minutes in concentrated sulphuric acid.

In this study, the high germination capacity and seedling emergence after seed desiccation during the warm stage of stratification suggest that this procedure enables very effective seed dormancy breaking, and that seed pre-treatment in citric acid solution or scarification in concentrated sulphuric acid are in fact alternative procedures. Seedling emergence in the second year after sowing the stratified seeds, may confirm the suggestions of some authors that cold-warm-cold stratification is necessary, with each phase lasting 2–3 months (Sheat 1948, Rushforth 1987, Scianna 2001). This may also indicate a large degree of adaptation of individual populations to unpredictable environmental conditions, although 3 days of seed desiccation between weeks 4 and 6 of the warm phase of stratification greatly reduced the values of seedling emergence in the second year.

The remarkable individual variation between seeds from various mother shrubs is probably due to the influence not only of environmental factors but seed ripeness and variations in genotype also. Seeds of shrubs A and B which grew on more fertile sites had a higher seedling emergence than seeds of shrub C, which grew on a very poor and dry site.

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