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Tadeusz Tylkowski

Seed germination and seedling emergence in *Hippophaë rhamnoides* L.

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Abstract: In contrast to earlier reports, in this study Seabuckthorn seeds collected from ripe fruits proved to be non-dormant. Most of them germinated quickly at high temperatures (e.g. cyclically alternating temperatures of 20~30°C daily for 16 and 8 hours, respectively). At lower temperatures (3~20°C), seeds did not germinate or germinated slowly, but stratification at 3°C for 4–6 weeks increased their germination rate to 90–100% within 2–3 weeks. Seedling emergence in the open nursery proved to be risky, even after 6–8 weeks of seed stratification, so spring sowing in the greenhouse (or plastic tunnel) is recommended.

Additional key words: seed dormancy; stratification; propagation from seeds; Seabuckthorn

Address: Polish Academy of Sciences; Institute of Dendrology; Parkowa 5,62-035 Kórnik, Poland, e-mail: ttylkows@man.poznan.pl

Introduction

Seabuckthorn (*Hippophaë rhamnoides* L.) belongs to the family Eleagnaceae, and in Poland it is a shrub or small tree up to 9 m high, while in Asia it reaches up to 18 m (Qin and Gilbert 2007). Its natural range of distribution is wide, including north-west Europe, central Asia to Altai Mountains, west and north China, north Himalaya Mts. (1600–3300 m), and in Tibet up to 5000 m. In Poland it grows wild on the Baltic coast, and its natural stands are legally protected. It does not need fertile soils, and colonizes barren dunes, forming dense thickets there. This species is resistant to drought stress, high temperature, and soil salinity. Because of its decorative silvery leaves and fruits with lovely yellow, orange or red orange colour at the end of summer, Seabuckthorn is willingly used in urban green areas, for strengthening of wayside slopes, and in reclamation of slag heaps or postindustrial grounds, or even as thorny "anti-vandal" hedges.

Being in symbiosis with diazotrophic bacteria (*Frankia* spp.), which live in root nodules, Seabuckthorn can use nitrogen from the atmosphere, converted into nitrogen compounds useful for plants (Hawker and Fraymouth 1951, Small and al. 2002, Kanayama and al. 2009).

Its fruits are berry-like achenes surrounded by fleshy hypanthium (Stace 1997). They are spherical or slightly elongate, 6–10 mm across, fleshy, with a characteristic smell and sour-acid taste. The fruits are rich in nutrients, such as vitamins, carotene, flavonoids, essential oils, carbohydrates, organic acids, minerals, etc. (Sabir et al. 2005). They are regarded as "superfruits", and are widely used as medicinal raw material (Zeb 2006) as well as in the cosmetic (Noculak-Palczewska & Rykowski 2003) and food industry, because of their culinary value (Dhyani et al. 2007).

In many countries, high-yielding cultivars have been developed and used in plantations (Werner 2002, Szałkiewicz & Zadernowski 2006, Bieniek et al. 2007). Environmental conditions in Poland are favourable for Seabuckthorn, which can prove to be an attractive alternative to traditional crops, and guarantee high profits to the producers (Giejbowicz & Wołek 2008).

Each fruit contains a single seed, which is slightly asymmetric. The average seed viability is 85%. One gram of clean and pure seeds contains 55–130 seeds, on average 88 (Slabaugh 1974).

Cram et al. (1960), Pearson & Rogers (1962), Dirr & Heuser (2006), and Gosling (2007) believe that mature seeds of Seabuckthorn are characterized by internal dormancy, which can be broken by seed stratification in sand for 90 days at 2–5°C. The germination test after stratification should be conducted at cyclically alternating temperatures of 20~30°C (Slabaugh 1974). According to Macdonald (1986) and Piotto & Di Noi (2003), seeds of this species should be sown in autumn, without any pre-treatment, or in spring after 4–12 weeks of cold stratification, but special attention should be paid to sowing them before they start to germinate during stratification. Seeds of this species can also be scarified chemically for 1 min in concentrated sulphuric acid before spring sowing.

This study was aimed to explain if Seabuckthorn seeds from the Polish population are dormant, and to determine optimum conditions for seed germination and seedling production.

Materials and methods

Two series of experiments were conducted. In 2001, experiments were intended to determine optimum conditions for seed germination in laboratory conditions and to assess the effect of stratification on their germination. In 2003, an efficient protocol for seedling production was developed.

Experimental series 1

Seabuckthorn seeds were extracted from fruits collected in the autumn of 2000, from 3 shrubs in Różyny near Gdańsk in northern Poland and in the Kórnik Arboretum in western Poland (Table 1). After separating seeds from the fleshy hypanthium, they were air-dried for 5–6 days at room temperature to a moisture content of 13.1–15.3%, and next stored in airtight containers at –3°C till mid-March 2001, i.e. till the beginning of the experiments (Table 1).

Germination tests were performed in the laboratory (3 replicates of 50 seeds each), directly after storage, and after 2, 4, 6, 8, and 10 weeks of stratification in a substrate at 3°C. The substrate used for stratification and germination tests was composed of sand and peat (fine quartz sand and sieved peat with pH 5.5–6.5, at a ratio of 1:1, v/v). Germination test was conducted at cyclically alternating temperatures of 3~20°C, 3~25°C, and 20~30°C, in daily cycles of 16 h at the lower temperature and 8 h at the higher temperature. After stratification for 24 weeks, germination tests were conducted only at 3~20°C.

During the tests, water in the substrate was replenished every week, whereas during cold stratification, it was done every 2 weeks. Germination was observed for up to 16 weeks. Seeds with radicles >2 mm long were regarded as germinated. After the germination tests, non-germinated seeds were dissected to distinguish healthy seeds from empty and decayed ones.

Results of experimental series 1 were analysed using Statistica software (1998).

Experimental series 2

In the experiments on seedling emergence, 4 seed batches from single shrubs were used. Two of them were collected in 2000, and the others in 2002 (Table 2). One of the shrubs grew in Różyny in northern Poland, while the others in Kórnik and Poznań in western Poland.

After storage in a cold store till March/April 2003, the seeds were next stratified without any substrate (i.e. moist seeds were kept at 3°C). The stratification consisted in soaking the seeds in water once a week in a plastic box for 1 hour, followed by straining them and storage of moist seeds in the same container in a cold store.

Because of too low temperature for emergence in the nursery during sowing time on May two periods of seed stratification were tested before: 6 and 8 weeks. Seeds from both variants were sown at the

Table 1. Characteristics of seeds used in experimental series 1

Seed batch No	Shrub A	Shrub B	Shrub C
Date and site of seed collection	13 Sep 2000 Różyny N 54°12’13” E 18°39’53”	13 Sep 2000 Różyny N 54°12’13” E 18°39’53”	3 Oct 2000 Kórnik Arboretum N 52°14’41” E 17°5’57”
Date of seed cleaning	15 Sep 2000	15 Sep 2000	3 Oct 2000
Date of seed drying	15–20 Sep 2000	15–21 Sep 2000	3–9 Oct 2000
Seed moisture content (%)	14.4	13.1	15.3
Period of seed storage at –3°C	20 Sep 2000–14 Mar 2001	21 Sep 2000–14 Mar 2001	9 Oct 2000–15 Mar 2001
Seed viability (TTC) after storage (%)	95.3	94.7	95.3

Table 2. Characteristics of seeds used in experimental series 2

Seed collection site	Różyny	Kórnik Arboretum	Kórnik Arboretum	Poznań, os. Oświecenia
Shrub No.	D	E	F	G
Seed collection date	13 Sep 2000	3 Oct 2000	2 Dec 2002	6 Dec 2002
Moisture content of seeds dried at room temperature	13.1%	14.0%	10.8%	11.8%
Seed storage at –3°C up to	24 Mar 2003 or 7 Apr 2003*			

*depending on planned stratification time (6 or 8 week)

same time. After stratification, germination tests were performed (i) in the laboratory in the sand-peat substrate, at cyclically alternating temperatures of 3~20°C (16+8 h daily). On 19 May 2003 stratified seeds were also sown: (ii) in plastic growing trays HIKO™ V-310 (tray dimensions: 353×213×100 mm; no. of cells/tray: 15; cells/m^2: 198; volume/cell: 310 cm^3), in a shaded plastic tunnel, and (iii) in the ground in a forest nursery of the Kórnik Arboretum at a depth of 1 cm, in 3 replicates of 50 seeds each. The seeds were sown in furrows impressed in the soil, next the seeds were covered with a 1-cm layer of sandy soil, and additionally with a 1-cm layer of milled, composted pine bark. The plastic cover of the tunnel was removed in early August. In the plastic tunnel, an automatic sprinkler was installed, while the plots in the nursery were sprayed manually, with a spray nozzle mounted on a hose. The nursery was not watered during weekends.

The growing trays were filled with a mixture of sphagnum peat (pH 5.5–6.5) with perlite No. 3 (at a ratio of 2:1, v/v). and the slow-release N-P-K fertilizer Osmocote was added, which releases nutrients for 5–6 months according to the manufacturer's instructions.

In the germination tests, the course of seed germination and germination capacity was assessed. In the nursery, all seedlings were counted after emergence and their height was measured at the end of the growing season. Results of this experimental series were not analysed statistically, because of their ambiguous interpretation.

Results

Experimental series 1

The germination tests show clearly that Seabuckthorn seeds are not dormant. When no stratification was performed, seeds from all batches at relatively high, cyclically alternating temperatures of 20~30°C or 3~25°C (16+8 h daily), started germination nearly immediately. At 20~30°C, all healthy seeds germinated within 2–3 weeks, while at 3~25°C, within 4–8 weeks, depending on seed batch. At 3~20°C, a lower percentage of seeds germinated (71.5% and 80.5%), and the germination tests lasted up to 16 weeks. Seeds of shrub C germinated at this temperature at the same level as at higher temperatures, but they needed 11 weeks to finish germination. These results indicate that the optimum temperatures for seed germination are within the highest range tested in the experiments.

If stratification was prolonged from 2 to 6 weeks, seed germination was markedly accelerated at all the tested temperatures. After 2-week stratification, seeds germinated on average within 2 weeks at 20~30°C, within 5 weeks at 3~25°C, and within 10 weeks at 3~20°C. After 4-week stratification, seeds germinated as early as within 1 week at 20~30°C, within 2 weeks at 3~25°C, and within 3 weeks at 3~20°C. After 6-week stratification, the germination of seeds of shrub B was delayed at 20~30°C, but accelerated at other temperatures. Further extension of stratification, to 8 and 10 weeks, caused a significant decrease in germination of seeds of 2 shrubs (A and B), while the percentage of seed germination for shrub C was still high. The lower germination rate of seeds of shrubs A and B resulted from seed decay during stratification or germination tests, as was proved by the dissection of non-germinated seeds after the tests. Seeds of shrub C did not react by a decrease in germinability after stratification prolonged to 24 weeks, in contrast to seeds of shrubs A and B, which germinated very poorly then (Table 3).

It is difficult to explain such a reaction to extension of seed stratification time. Perhaps the location of shrubs A and B on a slope near a road with heavy traffic and the toxic effect of the fumes emitted by vehicles, could decrease the quality of their seeds.

Experimental series 2

In laboratory conditions, the studied Seabuckthorn seeds germinated quickly. As early as in the first week of the germination test at 3~20°C, the first germinated seeds appeared, whereas by the end of the second week, already about 90% of seeds had germinated. Within 3 weeks, virtually all healthy seeds were germinated (Fig. 1).

Regardless of seed batch (Table 2), after 6–8 weeks of stratification without any substrate at 3°C, Seabuckthorn seeds sown in the plastic tunnel were characterized by very high emergence rates: 93.3–100% (Fig. 2). This was possible thanks to favourable weather conditions in 2003. The mean daily tempera-

Table 3. Seed erminability (%) and germination time of 3 seed batches (from shrubs A, B and C) during germination tests at 3~20°, 3~25°, and 20~30°C, depending on stratification time at 3°C. Culmination time of germination is given in brackets

Stratification time at 3°C (weeks)	% of seeds that germinated (and time of germination in weeks)								
	Shrub A			Shrub B			Shrub C		
	3~20°	3~25°	20~30°	3~20°	3~25°	20~30°	3~20°	3~25°	20~30°
0	80.5 fi	100 a	100 a	71.5 hk	100 a	100 a	99.0 ab	100 a	100 a
	(15)	(8)	(3)	(15)	(7)	(2)	(11)	(4)	(2)
2	100 a	100 a	100 a	88.0 bg	100 a	100 a	100 a	100 a	100 a
	(10)	(5)	(2)	(14)	(5)	(2)	(7)	(5)	(2)
4	94.5 af	100 a	100 a	96 ae	100 a	100 a	100 a	100 a	100 a
	(4)	(2)	(1)	(3)	(2)	(1)	(3)	(2)	(1)
6	94.0 af	95.5 ad	96.5 ad	88.5 ch	86.0 dh	98.0 ad	96.5 ad	98.0 ab	99.0 a
	(2)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(1)
8	49.5 kl	57.0 jl	62.0 il	74.5 gj	64.0 il	74.0 ei	98.5 ab	97.5 ac	96.0 ad
	(2)	(2)	(1)	(3)	(2)	(1)	(3)	(2)	(1)
10	42.0 l	45.5 l	45.0 l	52.5 jl	56.0 jl	44.5 l	98.0 ab	97.0 ad	97.5 ac
	(4)	(1)	(1)	(4)	(1)	(1)	(4)	(2)	(1)
24	2.0 m	–	–	3.0 m	–	–	88.5 ch	–	–
	(2)			(2)			(4)		

Different letters denote significant differences between treatments (P = 0.05, Tukey).

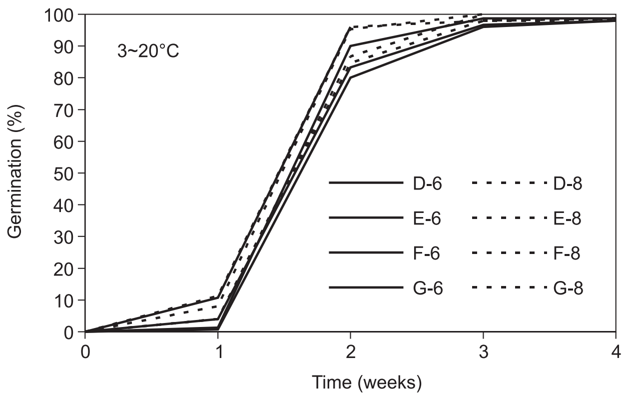

Fig. 1. Germination of seeds from shrubs: D, E, F and G, at cyclically alternating temperatures of 3~20°C, after stratification for 6 and 8 weeks

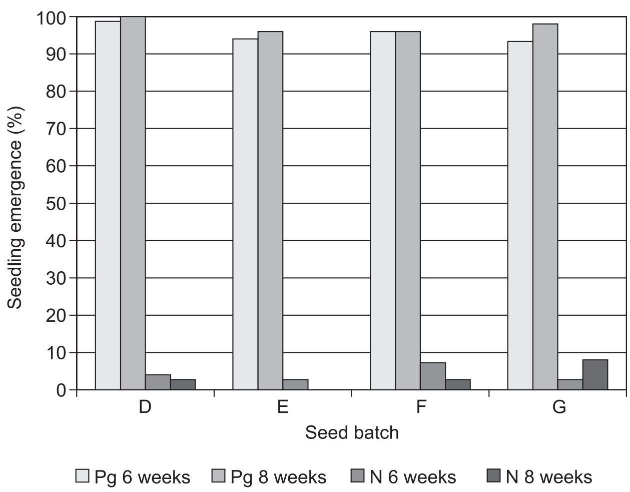

Fig. 2. Seedling emergence on 2 Jun 2003 (i.e. 2 weeks after sowing) in the plastic tunnel (Pg) and in the nursery (N) after sowing of seeds stratified for 6 and 8 weeks

ture measured in the meteorological box during the first two weeks after sowing of seeds not exceeded 17.5°C and the average temperature measured above ground in the same period was only 8.8°C. It is noteworthy that in the plastic tunnel, the temperature was always higher than outside. Experimental series 1 results show that the optimum temperature for Sea-buckthorn seed germination is relatively high and ranges from 20°C to 30°C.

The seeds sown in the nursery, after 6–8 weeks of stratification, were characterized by very low emergence rates (Fig. 2). Such poor results were probably due to excessive drought and lack of spraying, especially during weekends, and seed decay before seedling emergence, affecting germinating seeds.

Mean seedling height in the nursery, at the end of the growing season, differed between seed batches and ranged from 24.3 to 31.4 cm.

On some seedlings in the nursery male flower buds were noticed, i.e. their sex could be determined, which is normally possible only after the second or third growing season.

Discussion

Seed dormancy can be defined as the physiological state that does not enable the germination of healthy seeds left in untouched seeds coats in conditions that are favourable for germination (Bonner 1984, Bewley 1997, Leadem 1997). According to this definition, results of this study indicate that Seabuckthorn seeds are non-dormant.

At relatively low temperatures (3~20°C), seeds of this species germinate very slowly, while at relatively high temperatures (20~30°C), they germinate vigorously and reach a high germination rate, very much like seeds of Buckthorn (*Rhamnus cathartica*) (Tylkowski 2007). Cold stratification of Seabuckthorn seeds modified their germination pattern at cyclically alternating temperatures of 3~20°C, which resemble the natural soil conditions in spring. If stratification was prolonged from 2 to 6 weeks, then seeds germinated at this temperature much earlier than unstratified seeds. A similar positive influence of higher temperature was observed in seedling emergence in the plastic tunnel (Fig. 2).

In contrast to earlier recommendations (Bärtels 1982; Terpiński 1984), there is no risk of precocious seed germination during the stratification, because at 3°C the seeds did not germinate even after 24-week stratification, whereas their germinability decreased. Thus extension of Seabuckthorn seed stratification is not justified.

Stratification in a substrate can be replaced by stratification without any substrate, i.e. moist seeds can be simply kept at a low temperature, as this is sufficient to ensure a high seedling emergence rate. Thus the 3-month stratification of seeds before sowing, recommended by seed growers (Bärtels 1982; Terpiński 1984), can be shortened at least by a half.

Sowing in the nursery is rather risky, because Seabuckthorn seeds need relatively high temperatures for germination. In 2003, during the first 2 weeks after sowing, thermal conditions were unfavourable, so seedling emergence rates were very low. In contrast, seedling emergence in the plastic tunnel reached nearly 100%. Perhaps the nearly complete lack of seedling emergence in the nursery resulted from unfavourable weather conditions (drought and high temperature) and biotic factors (seed decay before seedling emergence).

In conclusion, this study showed that Seabuckthorn seeds collected from ripe fruits are non-dormant. Most of them germinated quickly at high temperatures (20~30°C). At lower temperatures (3~20°C), seeds did not germinate or germinated slowly. However, stratification at 3°C for 4–6 weeks increased germination rate at 3~20°C to 90–100% within 2–3 weeks. Seedling emergence in the ground (nursery) proved to be risky, so sowing in the greenhouse (or plastic tunnel) is recommended.

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