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Tadeusz Tylkowski

Seed storage, germination and seedling emergence in *Rhamnus catharticus*

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Abstract: This study was aimed to improve the understanding of germination ecology and to explain the invasive character of the common buckthorn (*Rhamnus catharticus*) in North America. Its fully mature seeds are characterized by a lack of dormancy. In laboratory conditions, favourable thermal conditions were identified for seed storage, germination and seedling emergence. At the cyclically alternating temperature of 20~30°C (16+8 h daily), seeds of this species showed a high germination rate within few weeks. Two other thermal variants, 3~15°C and 3~20°C (16+8 h daily), were much less effective for seed germination. However, cold stratification (at 3°C) in a moist mixture of peat and sand, lasting 4–8 weeks, caused a remarkable increase in germination rate also at 3~15°C. Seeds extracted from ripe fruits and dried (to a moisture content of about 10%) showed high germination and emergence rates after storage for 3.5 years at -3°C.

Additional key words: invasive species; landscape shrub; medicinal use

Address: Laboratory of Seed Biology, Institute of Dendrology, Polish Academy of Sciences
Parkowa 5, 62-035 Kórnik, ttylkows@man.poznan.pl

Introduction

The genus *Rhamnus* of the buckthorn family (Rhamnaceae) includes about 100 species of trees and shrubs. The scientific name of the genus derives from the Old Greek word *rhamnos*, which denotes a thorny bush.

The common buckthorn (*Rhamnus catharticus* L.) is a shrub or rarely a tree reaching up to 6 m in high, undemanding with respect to soil conditions, native to Europe from the Alps to southern Scandinavia and North Asia, up to the altitude of 1200 m.

In Poland it is common in lowlands, but less frequent in the submontane zone, preferring forest edges and thickets. In open habitats it provides protection for wildlife. It is a characteristic species for the class *Rhamno-Prunetea* and the association *Rhamno-Cornetum sanguinei*; as well as a distinguishing species for the community *Fagus sylvatica-Cypripedium calceolus* (Snowarski 2006).

Probably as early as in the 18th century, the common buckthorn was introduced to eastern North America (Wyman 1971), where it found particularly favourable conditions for growth and reproduction. Currently it constitutes there an important threat to old fields and has been classified as an invasive species, very much like the closely related alder buckthorn *Frangula alnus* (Gill and Marks 1991).

The shrub is densely branched, with thorns close to points of branching. Leaves - ovate or elliptic, 4–8 cm long and up to 3 cm wide, with arched veins. Flowers – small, greenish-yellow, in clusters of 2–5, opening in May. The shrubs can be dioecious, flowers unisexual or bisexual. Fruits – spherical (berry like drupe), covered with a black epidermis, ripening in late summer. Each fruit contains 2–4 nut like seeds (Hubbard 1974). The estimated 1000-seed weight is about 17 g. Bark and fresh fruits are poisonous. Fruits and bark contain also flavonoids and tannins, respectively (Internet 1). In the past, durable yellow, red and green pigments were extracted from its bark, fruits and leaves for the dyeing of fabric, leather, paper, and wood (Bugała 1991).

In nurseries this species can be propagated from seeds. According to Terpiński (1971, 1984) and Bärtels (1982), if its seeds are dried after collection and stored until spring, they need to be stratified for a year, because when sown in the nursery some seeds start to germinate after a year. By contrast, Tyszkiewicz (1952) suggests that buckthorn seeds, separated from the flesh of the fruit, should be sown in the nursery soon after collection in September, or else buried in fresh sand and sown in spring.

This study was aimed to determine favourable thermal conditions for seed germination and seedling emergence of fully mature seeds of the common blackthorn directly after their collection as well as after drying and storage. On the basis of obtained results, an attempt was made to explain what factors contribute to the invasive character of the common buckthorn in North America.

Material and methods

Fully ripe fruits were collected in 1996, separately from two shrubs marked as A and B (Table 1). Directly after collection, fresh seeds (stage 1) separated from the fruits squashed in water were subjected to cold stratification in a substrate at 3°C for 0, 4 and 8 weeks (3 replications of 50 seeds each, in plastic bottles of 0.25 l in volume). The substrate was a moist mixture of sieved peat (pH 5.5–6.5) with pure quartz sand (fraction <1 mm) at a ratio of 1:1. Optimum substrate moisture content was tested manually by squeezing in the hand – a single drop of water should leak between fingers (Gordon and Rowe 1982; Suszka et al. 1996) and checked every 2 weeks at 3°C.

After stratification, the seeds were subjected to germination tests at four different ranges of cyclically alternating temperatures of 20~30°C, 3~25°C, 3~20°C and 3~15°C (daily cycles of 16+8 h – i.e. 16 h at lower temperature and next 8 h at higher temperature per cycle). The germination tests were conducted in the same substrate. During the tests, seed germination was assessed weekly for 15 weeks (or shorter if germination finished earlier). During stratification and the germination tests, water was replenished if necessary and the seeds were aerated regularly.

Freshly harvested undried seeds (stage 1)

After collection, seeds from both shrubs, separated from the pericarp and dried at room temperature to the moisture content of 10.8% and 10.4% (Table 1), were also stored for 4–7 months, in tightly sealed containers at -3°C. Some seeds of shrub A were also stored in such conditions for 42 months. Moisture content (MC) of seeds as a percentage of water on a fresh weight basis was determined after their drying at the temperature of 105°C for 24 h.

Short-term storage of dry seeds (stage 2)

Seeds stored for 4–7 months were next stratified (4 replications of 50 seeds each) in the substrate for 3°C for 0, 4, 8 or 12 weeks. On 23rd April 1997, all germination tests and emergence tests were started and also seeds were sown directly in the nursery. Hence, in the variants with the longest stratification period, seeds were stored shorter, i.e., for only 4 months, while in the variant without stratification, seeds were stored the longest, i.e., for 7 months.

After stratification, the seeds were subjected to germination tests at 20~30°C and 3~25°C, and to the emergence test at 3~25°C (18+8 h daily). Seeds were also sown in a forest nursery in furrows pressed in the soil to the depth of 1 cm, and next covered with a sandy soil and a 3 cm layer of ground pine bark. The seedbeds with sown seeds were sprinkled with water to protect the soil from drying. These seedbeds were located in the eastern part of the nursery, so they were shaded in the morning by the adjacent forest stand. Emergence tests in the laboratory were carried out in plastic Stewart Unheated Propagators (trays: L 35 cm, W 21 cm, H 8 cm and crystal clear lid H 12 cm, with two adjustable air vents), after sowing in the stratification substrate at the depth of 1 cm.

Table 1. Characteristics of seed used for this study

Seed lot	Shrub A	Shrub B
Collection site 52°18'2" N; 17°1'47 E	Borówiec–Kamionki 200 m away from forest	Borówiec–Kamionki 300 m away from forest
Collection date	11 Sep'96	11 Sep'96
Moisture content of fresh seed (stage 1)	41,3%	41,2%
Moisture content of dried seed	10,8%	10,4%
Seed storage at -3°C (stage 2)	12 Sep'96–23 Apr'97 – 4–7 months	12 Sep'96–23 Apr'97 – 4–7 months
Seed storage at -3°C (stage 3)	12 Sep'96–15 Mar'00 – 42 months	–

Long-term storage of dry seeds (stage 3)

The seeds of shrub A that were stored at -3°C for 42 months were subjected to cold stratification at 3°C, and next to an emergence test (as in 1997) at cyclically alternating temperature of 3~25°C (16+8 h daily), and sown in a nursery on 10th May 2000 (4 replications of 50 seeds each).

Results of the seed germination tests conducted in laboratory conditions were subjected to an analysis of variance and tested with the Tukey T test at P=0.05 by the program Statistica (1997).

For a comparison of germination rates in various thermal conditions, the germination curves were used to estimate the "half-time" (t_{50}), i.e., the time needed to reach half of the maximum germination.

Results

Stage 1: freshly harvested undried seeds

Fresh, undried seeds of the common buckthorn are able to germinate without any previous stratification (Fig. 1) on condition that the temperature of germination is relatively high. Within the analyzed range of temperatures, the highest germination rates (86% of seeds of shrub A; 95% of seeds of shrub B) and lowest values of "half-time" (t_{50}=2.5 and 3.5 weeks, respectively; Table 2) were recorded at 20~30°C. At 3~25°C, germination proceeded much more slowly (t_{50}=6 and 11 weeks for seeds of shrub A and B, respectively), and germination rates were lower (45% and 22%, respectively) than at 20~30°C. Seeds of both shrubs directly after harvest (without any stratification) were unable to germinate at 3~20°C and 3~15°C.

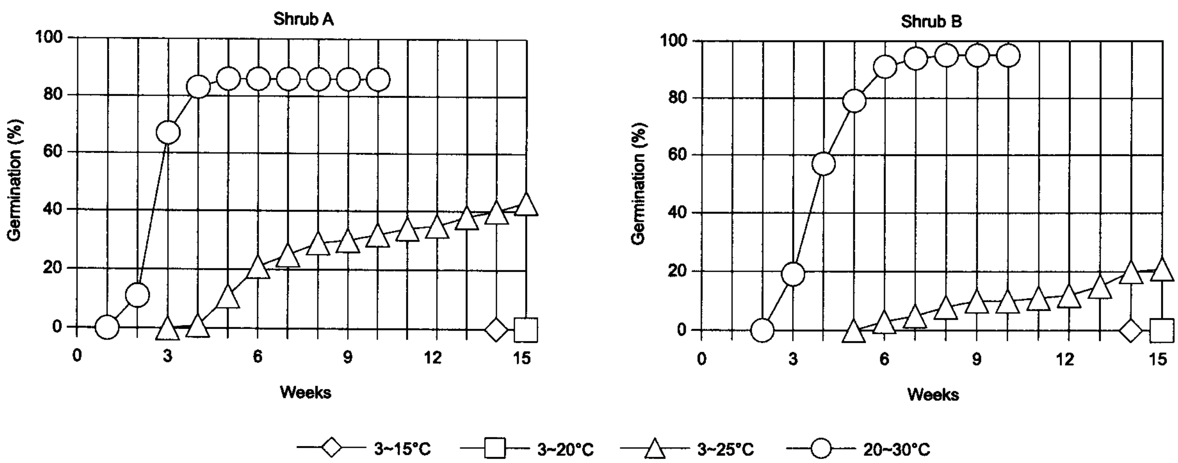

Fig. 1. Germination curves of fresh non-stratified seeds at 3~15°C, 3~20°C, 3~25°C and 20~30°C

Table 2. Germination rate of common buckthorn seeds (shrubs A and B) at various temperatures and their "half-time" of germination (t_{50} in weeks) depending on time of cold stratification (at 3°C) preceding the germination test. Values marked with the same letters within columns are not significantly different at P=0.05

Time of stratification	Temperature of germination	Shrub A		Shrub B	
		Germinability	t_{50}	Germinability	t_{50}
weeks	°C	%	weeks	%	weeks
0	3~15	0 f	-	0 f	-
0	3~20	0 f	-	0 f	-
0	3~25	45 de	6.0	22 e	11.0
0	20~30	86 abc	2.5	95 ab	3.5
4	3~15	26 e	12.5	0	-
4	3~20	81 abc	9.5	37 de	11.0
4	3~25	91 ab	5.2	88 abc	7.0
4	20~30	93 ab	1.5	95 ab	2.0
8	3~15	63 cd	9.5	32 de	13.0
8	3~20	85 abc	5.0	77 bc	7.0
8	3~25	87 abc	3.5	89 ab	4.5
8	20~30	97 a	1.5	97 ab	2.0

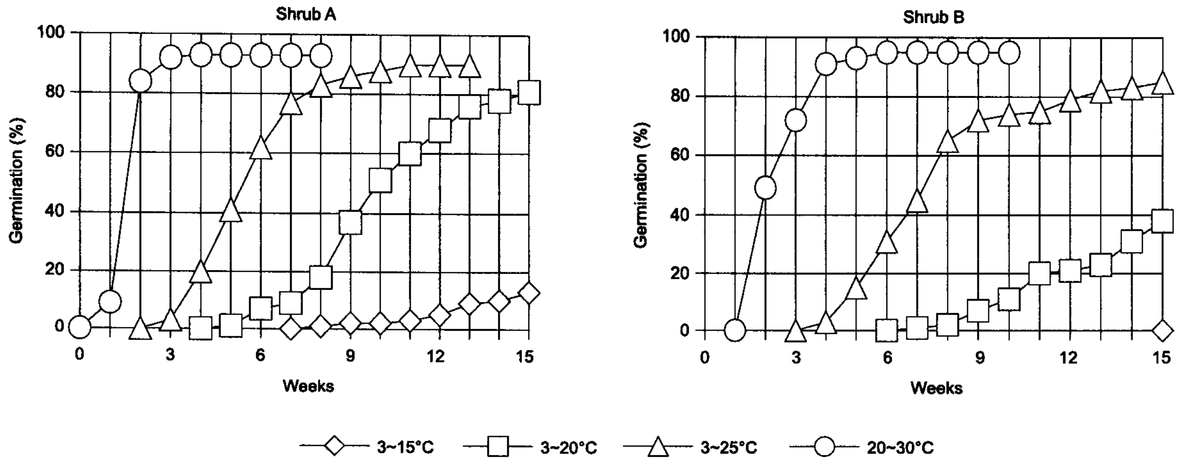

Fig. 2. Germination curves of fresh seeds at 3~15°C, 3~20°C, 3~25°C and 20~30°C after 4 weeks of cold stratification at 3°C

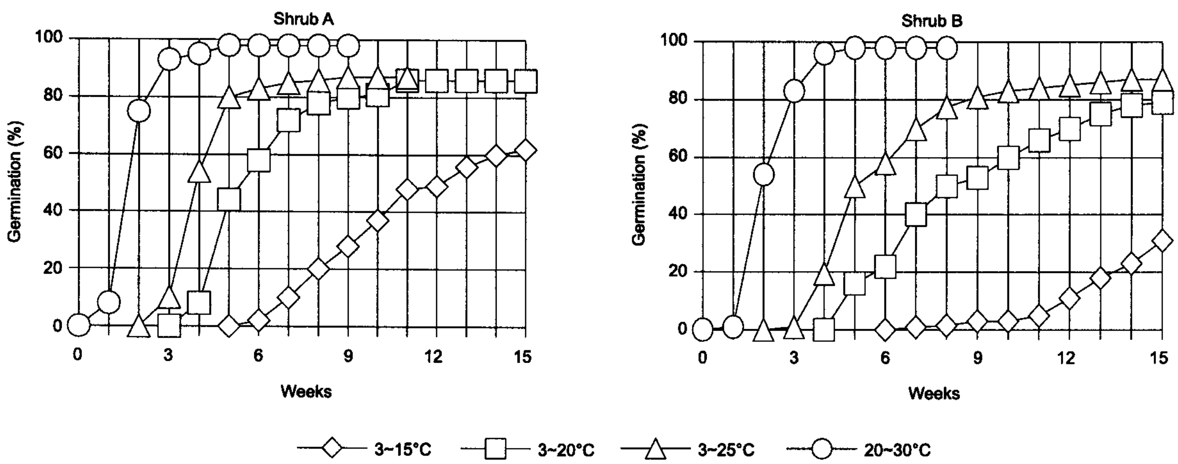

Fig. 3. Germination curves of fresh seeds at 3~15°C, 3~20°C, 3~25°C and 20~30°C after 8 weeks of cold stratification at 3°C

However, after 4 weeks of cold stratification (at 3°C) of the undried seeds, their germination at 20~30°C was greatly accelerated (t_{50}=1.5 and 2 weeks for seeds of shrubs A and B, respectively). Also at 3~25°C the "half-time" improved remarkably then (t_{50}= 5 and 7 weeks, respectively), and germination rates increased to 91% and 88% for seeds of shrubs A and B, respectively. After 4 weeks of stratification, seeds germinated also at 3~20°C, although the germination rates were low (Fig. 2). Germination was also observed at 3~15°C, but only in the case of seeds of shrub A, while seeds of shrub B did not germinate in that thermal variant.

The stratification prolonged to 8 weeks stimulated germination at 3~25°C even more strongly, which was then markedly faster, as reflected in the value of t_{50}=4 weeks (Fig. 3).

Stage 2: short-term storage of dry seeds

After storage for 4–7 months at -3°C, the seeds still were characterized by high rates of germination and seedling emergence (Table 3 and 4). The highest percentage of unstratified seeds of shrub A germinated at 20~30°C and a somewhat lower percentage at 3~25°C, but those values did not differ significantly at P=0.05 (Table 3). Seeds of shrub B germinated more uniformly irrespective of the thermal variant but their results did not differ significantly from those of shrub A (Table 3).

Under laboratory conditions, irrespective of the duration of stratification (0, 4 or 8 weeks) no significant variation in seedling emergence was observed between the shrubs (Table 4). In the nursery, however, a significant variation in seedling emergence was observed between the two shrubs. Seedling emergence from unstratified seeds reached 9% for shrub A, but 0% for shrub B (Table 4). After stratification for 4 or 8 weeks, seedling emergence was significantly higher for seeds of shrub A than for seeds of shrub B. In general, emergence rate was significantly higher for seeds of shrub A than for seeds of shrub B.

Seedling height in the nursery after the first growing season was relatively low, and the mean was 23.8 mm. The largest seedlings did not exceeded 35 mm in height.

Table 3. Germinability of common buckthorn seeds (shrubs A and B) and their "half-time" of germination (t_{50}) after storage at -3°C for 4–7 months. The seeds were stratified at 3°C for 0, 4 or 8 weeks and next subjected to germination tests at 3~25°C and 20~30°C

Time of stratifi-cation	Germinability (%) and "half-time" of germination (t_{50})							
	Shrub A				Shrub B			
	20~30°C	t_{50}	3~25°C	t_{50}	20~30°C	t_{50}	3~25°C	t_{50}
weeks	%	weeks	%	weeks	%	weeks	%	weeks
0	90 a	2.5	82 ab	8	87 ab	4.5	85 ab	11
4	83 ab	2	80 ab	5.5	89 a	3.5	82 ab	8
8	74 b	2	76 ab	6	84 ab	3	82 ab	7

Table 4. Seedling emergence from seeds of two shrubs in the laboratory at 3~25°C and in the nursery. Seeds after storage for 4–7 months at -3°C were stratified at 3°C for 0, 4 or 8 weeks. Sowing date: 23rd April 97

Duration of stratifi-cation	Emergence rate					
	Shrub A			Shrub B		
	3~25°C	t_{50}	nursery	3~25°C	t_{50}	t_{50}
weeks	%	weeks	%	%	weeks	weeks
0	84 a	7	9 e	72 ab	8.5	0 f
4	90 a	7.5	42 c	88 a	10	15 de
8	85 a	7	51 bc	86 a	8	32 cd

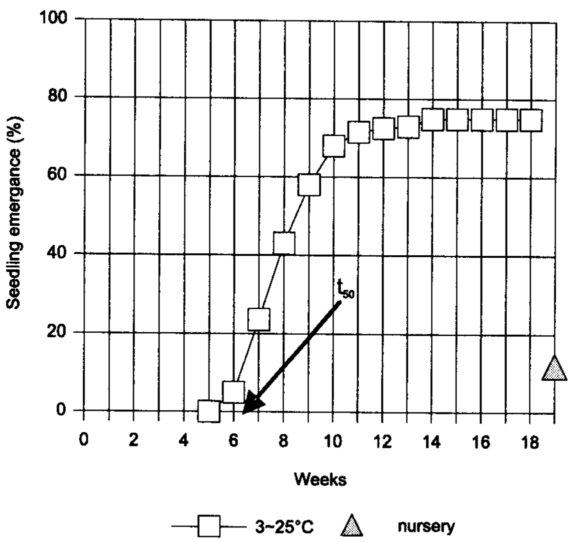

Fig. 4. Seedling emergence from seeds at 3~25°C after 8 weeks of stratification at 3°C, compared with seedling emergence in the nursery. An arrow indicates "half time" of seed germination (t_{50})

Stage 3: long-term storage of dry seeds

Seeds dried after harvest and stored for 42 months (at -3°C), and next subjected to cold stratification (at 3°C) for 8 weeks, showed an emergence rate of 75% after 14 weeks in laboratory conditions at 3~25°C, compared to only 12% in the nursery (Fig. 4).

Discussion

The common buckthorn has been introduced to North America because it thrives on less fertile soils and can be planted both in hedges and in the wild. In the USA, the species has quickly escaped from cultivation and currently in the North-East it widely colonizes wetlands, forest edges, former farmland, prairies, ravines, etc., where it competes with native plant species (Taft and Solecki 1993). Common buckthorn bushes also pose a threat to soybean fields, because in winter the bushes are inhabited by soybean aphids (*Aphis glycines* (Takashi et al. 1993; Ostile 2001), and also to oats, because buckthorn is an intermediate host of oat rust (Ginns 1986).

It seems that one of the reasons for the invasiveness of the common buckthorn in North America can be the high thermal requirements of seeds of this species during germination. In spring both precipitation and soil temperature are in some regions probably higher than in Poland. Also the meridional orientation of mountain ranges is favourable, because it facilitates the movement of warm and moist air masses from the south.

Results of this study showed that buckthorn seeds germinated and emerged much easier and faster at the highest range of the applied temperature, e.g., 20~30°C, than at lower temperatures, e.g., 3~25°C (16+8 h daily). Fresh undried seeds sown directly after harvest were unable to germinate at 3~15°C and 3~20°C. After several weeks of stratification in a substrate at 3°C, the seeds were capable of germination at both the lower thermal variants, at a relatively high rate, which increased even further if cold stratification was prolonged. Also seeds dried after harvest to a moisture content of about 10% and stored for 42 months at -3°C were afterwards able to germinate and emerge in the nursery.

In Polish climatic conditions, Tyszkiewicz and Dąbrowska (1953) found that when whole fruits soon after harvest were stratified in natural conditions (in a hole in the ground at a depth of 40–47 cm, covered with a 25-cm layer of sand and a 10-cm layer of compacted leaf mould), then the pericarp did not pose any obstacle to seed germination and seedling emergence the following spring.

Hubbard (1974) recommended that before sowing, the seeds should be subjected to scarification in sulphuric acid for up to 20 minutes or to cold stratification (at 1–5°C) for 6 months, followed by a germination test at cyclically alternating temperatures of 20~30°C (18+6 h daily). However, in the light of the research presented in this paper, neither scarification nor such a long cold stratification are needed.

In natural conditions the seeds that over winter on the surface of the ground (in rotten fruits below bushes or after defecation by birds) are exposed to humidity and cold for a sufficiently long time to show in spring very high germination and emergence rates. If environmental conditions during seedling emergence are favourable for rooting and further growth, this may be sufficient to explain the high invasiveness of buckthorn in North America.

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