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Dormancy breaking, germination, and seedling emergence from seeds of *Crataegus submollis*

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Abstract: Effects of several stratification variants on seed dormancy breaking were compared in *Crataegus submollis* Sarg. (hairy cockspur-thorn or Quebec hawthorn). Ripe seeds were collected (in October), cleaned, and dried to a moisture content of 7–12%. Seed dormancy in this species was broken most effectively by warm-cold stratification of nutlets, in a substrate or without any substrate, at 15~25°/3°C or 20~30°/3°C, i.e. with a cyclically alternating warm stage (16+8 hrs or 24+24 hrs/cycle) lasting 16–20 weeks, followed the cold stage lasting ca. 20 weeks, i.e. till the appearance of the first germinating seeds. After stratification, emergence rate is equally high (ca 50%) at cyclically alternating temperatures of 3~15°C and 3~20°C (16+8 hrs). Chemical scarification of nutlets in 96% sulphuric acid for 3 hrs, followed by warm-cold stratification at 20~30°/3°C, with a short, 4-week warm stage, also ensures a high emergence rate (58%). Seed desiccation (in nutlets) slowly to a moisture content of 10–12%, after stratification in a substrate or without any substrate as well as after scarification, results in a reduced emergence rate, especially if seeds are dried to the lower moisture content. Seed storage (in nutlets after drying to a moisture content of 10%) for 10 years at –3°C, does not decrease the emergence rate (93%) after stratification at 20~30°/3°C in a substrate, with a cyclically alternating warm stage (24+24 h) lasting 16 weeks.

Additional key words: stratification, scarification, germination, seedling emergence, desiccation, storage

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Introduction

Seeds of hawthorns, as of many other tree and shrub species in the temperate zone, are characterized by dormancy. The basic procedure enabling dormancy breaking and thus germination is seed stratification in controlled conditions. In the case of physical dormancy of seeds (impermeability of the seed coat or the pericarp) also scarification is helpful: mechanical, in boiling water, or chemical (Suszka et al. 1994).

Seed dormancy in hawthorns is combinational: both physical (due to the presence of a hard endocarp and seed coat) and physiological (Tyszkiewicz and Dąbrowska 1953; Nikolaeva 1967; Lang et al. 1987; Hartmann et al. 1997; Baskin and Baskin 2004).

The genus *Crataegus* belongs to the family Rosaceae, subfamily Maloideae, which includes very numerous and variable species. The great polymorphism is caused by hybridization, polyploidization and apomixis (Dickinson et al, Phipps 1988; Radford et al. 1968; Robertson 1991; Vines 1960). In the systematic classification of the genus *Crataegus* suggested by Seneta and Dolatowski (2000), two subgenera are distinguished: *Crataegus* and *Americanae,* and the latter includes *C. submollis*, which is the subject of this study. Currently it is estimated that the number of hawthorn species in North America is between 100 and 200 (Christensen 1992; Kalkman 2004; Phipps et al. 1990, 2003).

Hairy cockspur-thorn or Quebec hawthorn (*C. submollis* Sarg., syn. *C. champlainensis* Sarg.) is distributed in northeastern North America: in Quebec Ontario, Massachusetts, and New York. In Poland it is an introduced species, commonly planted in parks (shrubs 3–4 m high), singly or in hedgerows. It flowers abundantly in May, with white, scented blossoms, whereas fruit is large, red, with 3–4 nutlets. Its flowers and fruit are used in medicine to reduce blood pressure (Moerman 1998; Moore et al. 1986; McMillan-Browse 1985; Yang and Yang 1992).

The aims of this study were: (1) to determine optimum conditions of seed dormancy breaking (stratification and/or chemical scarification); (2) to assess the influence of seed storage on dormancy breaking; and (3) to identify the optimum temperature of germination and seedling emergence after dormancy breaking.

Material and methods

Seeds (in nutlets) of one provenance, collected in various years when fruits were ripe, were used in the experiments as fresh, dried, or stored (Table 1). After cleaning, the seeds were subjected to a viability test by cutting and their moisture content was estimated (105°C, 24 h). Seeds were next subjected to warm-cold stratification in a substrate (sand and peat 1: 1, pH 3.5–4.5) or without any substrate (only soaked in water for 1 h once a week) and/or to chemical scarification in concentrated sulphuric acid for 1, 2 and 3 h. After scarification, the nutlets were moved to a sieve, thoroughly rinsed in tap water, and soaked in water for ca. 20–24 h. After stratification and/or scarification, the seeds were dried slowly (in a cold store at 3°C for 6 days and next in an air current for 1 h at room temperature). At the end of stratification, the germination and emergence tests were performed in 3 replications of 50 seeds each, in the same substrate. Detailed information on the applied thermal conditions of stratification in the substrate or without any substrate and/or scarification, as well as temperatures of germination and emergence tests, was given in captions to tables and figures. At the end of the stratification, ungerminated seeds were dissected, and the results were calculated as percentage of full seeds. Germination and seedling emergence were subjected to analysis of variance (ANOVA) after arc-sin transformation. The significance of results was assessed by Tukey test at $P \leq 0.05$. For the analyses, JMP 4.0.2 software was used.

Results

Stratification in a substrate or without any substrate

In Experiment I (seed lot 1) germination and seedling emergence were the highest for the seeds that after collection were dried to a moisture content of 9.2%, after warm-cold stratification 15~25°C (16+8 h) and a cold stage at 3°C for 20 weeks (Table 2).

In Experiment II (seed lot 1 a) germination and seedling emergence were the highest after warm-cold stratification, with the warm stage at 15~25°C (16+8 h for 20 weeks), on average 38.5% and 43.0%, respectively. Prolongation of warm stratification to 20 weeks caused an increase in the seedling emergence if the warm stage of stratification was at 15~25°C (16+8 h) (Table 3).

In Experiment III (seed lot 2), we compared the effectiveness of seed pretreatment by warm-cold stratification at 15~25°/3°C (16+8 h or 24+24 h; Table 4). Germination was the highest (mean 53%) after warm-cold stratification at 15~25°/3°C, if temperatures in the warm stage alternated every 24 h. Prolongation of the warm stage to 24 weeks proved to be ineffective (Table 4).

In Experiment IV (seed lot 3), where the warm-cold stratification was conducted at 30°/3°C, 20~30°/3°C and 15~25°/3°C (with the cyclically alternating temperature in the warm stage 24 + 24 h), the highest seedling emergence (49%) was recorded after warm-cold stratification at 20~30°/3°C (Table 5).

Experiment V (seed lot 3) showed that differences in the warm stage of stratification in a substrate or without any substrate at 15~25°/3°C (16+8 h or 24+24 h) did not have any effect on seed germination.

Table 1. *Crataegus submollis* Sarg. Characteristics of seed lots

No. of seed lot	Provenance	Year of seed collection	Moisture content of seeds after drying	Seed storage at -3°C		Viability of seeds (cutting test)
				time	moisture content after storage	
			%	years	%	%
1	Kórnik	2000	9.2	0	–	80.0
1a	Kórnik	2000	9.2	1	13.2	80.0
2	Kórnik	2001	12.7	0	–	70.0
3	Kórnik	2002	10.0	0	–	56.0
4	Kórnik	1994	10.0	8	11.0	58.0
5	Kórnik	1992	7.0	10	7.5	47.0

Table 2. *Crataegus submollis* Sarg. Germination and seedling emergence in the laboratory from undried seeds and from seeds dried after collection, in various variants of warm-cold stratification. Means marked by the same letters do not differ significantly (Tukey test at $P \leq 0.05$). Experiment I, seed lot 1

Moisture content of fresh seeds	Warm stage 16 weeks	Cold stage at 3°C	Germination (%) in thermal variants				Seedling emergence (%)
%	°C	weeks	3°C	3~15°C	3~20°C	3~25°C	3~20°C
	25	22	13 c	15 c	15 c	14 c	16 c
19.3	20~30	22	29 ab	25 b	25 b	22 b	28 b
	15~25	22	36 a	35 a	36 a	34 a	40 a
Mean			**26.0**	**25.0**	**25.2**	**23.3**	**28.0**
	25	23	7 c	9 c	9 c	10 c	9 c
9.2	20~30	23	19 b	18 b	21 b	23 b	20 b
	15~25	20	41 a	40 a	38 a	40 a	42 a
Mean			**22.3**	**22.3**	**22.7**	**24.3**	**23.6**

Table 3. *Crataegus submollis* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate. Means marked by the same letters do not differ significantly (Tukey test at $P \leq 0.05$). Experiment II, seed lot 1a

Thermal variant of stratification			Germination at 3°C	Seedling emergence at 3~20°C
Warm stage		Cold stage		
°C	weeks	weeks	%	%
12.5	16	20	3 c	3 c
	20	23	7 c	13 c
Mean			**5.0**	**8.0**
15	16	20	29 b	22 b
	20	22	28 b	24 b
Mean			**28.5**	**23.0**
20	16	20	24 b	27 b
	20	22	18 bc	20 bc
Mean			**21.0**	**23.5**
15~25 (16+8 h)	16	20	34 b	44 a
	20	22	43 a	42 a
Mean			**38.5**	**43.0**

Table 4. *Crataegus submollis* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate, at 15~25°/3°C with differences in the warm stage (Tukey test at $P \leq 0.05$). Experiment III, seed lot 2

Warm stage		Cold stage at 3°C	Seedling emergence at 3~20°C
°C	weeks	weeks	%
15~25 (16+8 h)	16	25	45 ab
	20	26	41 ab
	24	26	37 c
Mean			**41.0**
15~25 (24+24 h)	16	25	52 a
	20	26	56 a
	24	26	52 a
Mean			**53.0**

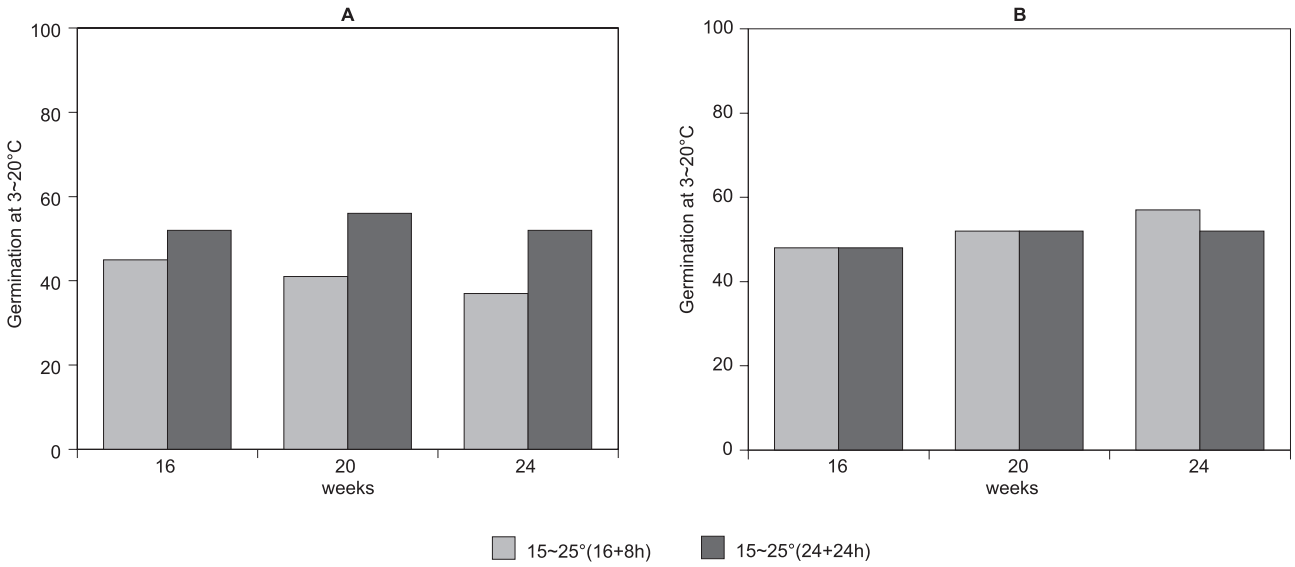

Fig. 1. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate (**A**) or without any substrate (**B**) at 15~25°/3°C, with the warm stage lasting 16, 20 or 24 weeks and the cold stage lasting 21–25 weeks. Experiment V, seed lot 3

Table 5. *Crataegus submollis* Sarg. Comparison of effectiveness of seed pretreatment after warm-cold stratification in a substrate (Tukey test at $P \leq 0.05$). Experiment IV, seed lot 3

Warm stage 16 weeks	Cold stage at 3°C	Seedling emergence at 3~20°C
°C	weeks	%
30	23	24 c
20~30	20	49 a
15~25	20	30 b

Germination was similar in both variants: on average 52.3% and 50.7%, respectively. Prolongation of the warm stage to 24 weeks proved to be useless (Fig. 1).

Chemical scarification of seeds

Chemical scarification was tested in one seed lot (3).

Seedling emergence from seeds of *C. submollis* at 3~20°C was the highest (58%) when the nutlets were scarified chemically for 3 h and next subjected to warm-cold stratification, with a 4-week warm stage at 20~30°C and a 19-week cold stage at 3°C. The shorter time of scarification (1–2 h) proved to be less effective. For unscarified seeds, after stratification at 20~30°C for 16 weeks and cold stratification at 3°C, emergence rate reached 49% (Fig. 2).

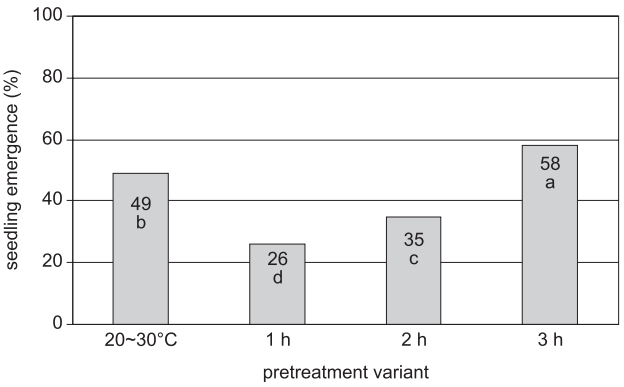

Fig. 2. Seedling emergence at 3~20°C (16+8 h) after warm-cold stratification at 20~30°/3°C (16+20 weeks) and scarification in 96% sulphuric acid for 1, 2 and 3 h, or after warm-cold stratification at 20~30°/3°C (4+19 weeks). Experiment VI, seed lot 3

Effect of drying of seeds after stratification in a substrate and/or after scarification

In Experiment VII (seed lot 4), after warm-cold stratification in a substrate at 15~25°/3°C, 20~30°/3°C (24+24 h/cycle) and 30°C, seedling emergence at 3~20°C was low (mean 35.3%, with an optimum in the variant with the warm stage at 20~30°C, i.e. 52%). Slow drying of stratified seeds to a moisture content of 10.8–12.3% caused a remarkable decrease in seedling emergence i.e. to 5.3% (Table 6).

In Experiment VIII (seed lot 5), slow drying of seeds after warm-cold stratification with the variable warm stage at 15~25°, 20~30°C (24+24 h/cycle) or at a constant temperature of 30°C, caused a decrease in emergence rate. The mean emergence rate of undried seeds was 79%, while that of dried seeds was 67.7% (Table 7).

In Experiment IX (seed lot 3), slow drying of seeds after chemical scarification had a negative effect on seedling emergence. Emergence rates were the highest (58%) after chemical scarification for 3 h, followed by warm-cold stratification with a 4-week

Table 6. *Crataegus submollis* Sarg. Seedling emergence at 3~20°C (%) after warm-cold stratification in a substrate and after drying. Experiment VII, seed lot 4

Thermal variant of stratification		Treatment after stratification		
Warm stage 16 weeks	Cold stage at 3°C	Seeds not dried	Seeds dried	
°C	weeks			
15~25		33.0	6.0	(12.3%)
20~30	22	52.0	8.0	(10.9%)
30		21.0	2.0	(10.8%)
Mean		**35.3**	**5.3**	(11.3%)

Table 7. *Crataegus submollis* Sarg. Seedling emergence at 3~20°C (%) after warm-cold stratification in a substrate and after drying (Tukey test at $P \leq 0.05$). Experiment VIII, seed lot 5

Thermal variant of stratification		Treatment after stratification		
Warm stage 16 weeks	Cold stage at 3°C	Seeds not dried	Seeds dried	
°C	weeks			
15~25		69.5 bc	68.0 bc	(11.7%)
20~30	18	93.0 a	64.0 c	(11.6%)
30		74.5 b	71.0 b	(12.2%)
Mean		**79.0**	**67.7**	**(11.8%)**

Table 8. *Crataegus submollis*. Seedling emergence at 3~20°C (%) after chemical scarification, warm-cold stratification and/or drying. Experiment IX, seed lot 3

Seed pretreatment			Treatment after stratification		
96% sulphuric acid	Warm stage 20~30°C	Cold stage at 3°C	Seeds not dried	Seeds dried	
h	weeks	weeks			
1			26.0	1.0	(8.8%)
2	4	19	35.0	2.0	(9.9%)
3			58.0	0.0	(10.0%)

warm stage at 20~30°C (24+24 h/cycle) and a cold stage at 3°C, while drying to a moisture content of ca. 10.0% after stratification reduced emergence rate to 0–2% (Table 8).

Long-term seed storage

In Experiment X (seed lot 5), after seed storage (in nutlets) with a moisture content of 7.0% for 10 years at -3°C, and seed pretreatment by warm-cold stratification at 20~30°/3°C (24+24 h/cycle), seed viability was relatively low (Table 1). However, from full seeds, seedling emergence at 3~20°C (16+8 h daily) were very high (93%) (Fig. 3).

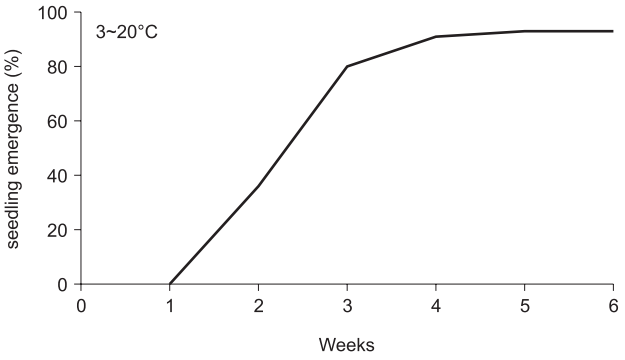

Fig. 3. *Crataegus submollis* Sarg. Seedling emergence at 3~20°C (16+8 h daily) after warm-cold stratification at 20~30°/3°C (16+15 weeks). Experiment X, seed lot 5

Discussion

Other authors like McMillan-Browse (1985), Sheat (1948), Thompson and Morgan (1990), recommended warm-cold stratification of *C. submollis* Sarg. seeds at 15°C for 3 months and at 4°C for another 3 months. The effectiveness of such a thermal variant of seed pretreatment was compared in this study with other variants: 12.5°/3°C, 20°/3°C, 30°/3°C, and cyclically alternating temperatures of 15~25°/3°C or 20~30°/3°C, with the warm stage cycle of 16+8 h daily or 24+24 h/cycle for 16, 20, or 24 weeks, and the cold stage at 3°C lasting ca. 20–26 weeks, i.e. till the appearance of first germinating seeds. Our results showed that seed dormancy in this species is broken most effectively by warm-cold stratification in a substrate, at 15~25°/3°C or 20~30°/3°C, with the cyclically alternating warm stage (24+24 h/cycle) lasting 16 weeks, and a cold stage lasting till the appearance of the first germinating seeds. The thermal conditions recommended by the authors mentioned above were less effective.

Our results indicate that seeds of *C. submollis* Sarg. can be successfully pretreated by stratification without any substrate, if the most favourable thermal variants of warm-cold stratification are applied. Such seed pretreatment in this species, not applied earlier, should be verified with the use of a large number of seed lots.

One of the methods increasing the permeability of the seed coat (and/or endocarp) is their chemical scarification with 96% sulphuric acid (St-John-S 1982). This method is recommended also for seeds of *C. submollis* Sarg. (McMillan-Browse 1985, Felipe et al. 1989) and enables shortening the time of warm-cold stratification, which is an advantage of this method. However, its disadvantage is the need to determine precisely the conditions of scarification without an excessive rise in temperature, which would be dangerous for seeds (Tylkowski 2000).

In our study, scarification (for 1, 2 or 3 h) was followed by a short, 4-week warm stage of stratification and cold stratification at 3°C, so this method was considered more effective, on the basis of previous research on chemical scarification of *C. monogyna* Jacq. and published literature (Piotto 2002).

Dormancy breaking after stratification and/or scarification can be delayed by partial dehydration of seeds. This can be utilized in practice if it is necessary to postpone the sowing date, or if bad weather at the beginning of seed germination does not enable immediate sowing. This method makes it also possible to store nondormant seeds (after dormancy breaking) and to offer them to nursery owners.

After slow drying to a moisture content of 9–13%, emergence rates of *C. submollis* Sarg. seeds in the laboratory were always lower than those of undried seeds (Table 6, 7). However, this method needs to be tested more precisely.

At –3°C, *C. submollis* Sarg. seeds were stored for 10 years, and after dormancy breaking their emergence rate reached 93%. Apparently nobody succeeded to store viable hawthorn seeds for such a long time before. These results form the basis for long-term seed storage of this species.

Conclusions

a) Seed dormancy in *C. submollis* Sarg. can be effectively broken by warm-cold stratification of nutlets, in a substrate or without any substrate, at 15~25°/3°C or 20~30°/3°C, with the cyclically alternating warm stage (16+8 h daily or 24+24 h/cycle) lasting 16–20 weeks and the cold stage lasting ca. 20 weeks,

b) After stratification, emergence rate is equally high (mean 50%) at cyclically alternating temperatures of 3~15°C or 3~20°C (16+8 h daily). For only one seed lot (5) emergence rate was very high (93% of full seeds) after stratification at 20~30°/3°C in a substrate, with a cyclically alternating warm stage (24+24 h/cycle) lasting 16 weeks. However, only 47% of seeds in that seed lot were full.

c) Chemical scarification of nutlets in 96% sulphuric acid for 3 h, followed by warm-cold stratification at 20~30°/3°C with a short, 4-week warm stage, also ensures a high emergence rate (58%). Longer time of scarification (3 h) had a positive effect on germination and emergence rates.

d) Seed drying (in nutlets) slowly to a moisture content of 12%, after stratification in a substrate, did not reduce emergence rates. Drying of nutlets to a moisture content of ca. 10%, after scarification and/or stratification, caused a remarkable decrease in emergence rates.

e) Seed storage (in nutlets, after drying to a moisture content of 7,5%) for 10 years at -3°C, did not decrease the emergence rate (93%).

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