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Seed dormancy breaking in *Crataegus laevigata*

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Abstract: Laboratory experiments were made to determine the optimum conditions for dormancy breaking in the midland hawthorn (*Crataegus laevigata* (Poir.) DC. = *C. oxyacantha* L.). Its small applelike fruits should be collected when they are fully ripe (in Poland in October). The nutlets extracted from the fruits must be dried at room temperature to the moisture content of 9–13%. The dormancy of midland hawthorn seeds can be overcome by: (1) stratification in a moist medium: 20~30°C/3°C, 16–20 weeks at 20~30°C (16+8 hrs or 24+24 hrs) followed by 16–18 weeks at 3°C, i.e. to the time when first radicles start to appear; or (2) chemical scarification in concentrated sulphuric acid for 2 or 3 hrs, followed by warm stratification at 27.5°C or 20~30°C for 4 weeks and cold stratification at 3°C, lasting 19–21 weeks, i.e. to the time when first radicles start to appear. The stratified seeds germinate vigorously (in 3–5 weeks) and at a high percentage at temperatures of 3~15°C or 3~20°C (16+8 hrs) and all seedlings emerge in such conditions about 4–6 weeks after sowing. Seed germination after stratification or scarification can be stopped by partial desiccation of seeds. Seed desiccation after stratification to the moisture content of 10–13% and sealed storage at –3°C for one year do not reduce seed germination and seedling emergence rates of the previously pretreated seeds. Storage for 20 months at –3°C of seeds dried after harvest to the moisture content of 14% does not reduce their germination and seedling emergence.

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

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Introduction

Seeds of numerous tree and shrub species require specific environmental conditions to initiate germination. These include appropriate temperature, air access and humidity. Seeds of many hawthorns exhibit double dormancy: endocarp and embryo dormancy (Tyszkiewicz and Dąbrowska 1953; Nikolaeva 1967; Lang et al. 1987; Hartmann et al. 1997).

The genus *Crataegus* belongs to the subfamily *Maloideae* in the family *Rosaceae,* a group of complex genera able to interbreed freely (or hybridize), as they all have the basal chromosome number of 17 (Phipps et al. 1990, Robertson et al. 1991, Mirek et al. 2002). They are native to the Mediterranean region including North Africa and all of Europe and Central Asia, but also to many areas of North America. Due to hybridization, more than 1000 species of hawthorns have been distinguished, but many of them are not regarded as valid now appear in more than a thousand different species. Recently, taxonomists have recognized in North America 100 to 200 species (Phipps et al. 1990).

The midland hawthorn (*Crataegus laevigata* = *C. oxyacantha*) grows in forests, where it is a valuable biocoenotic admixture, as its fruits are eaten by many bird species (Bugała 1991). In Poland the species occurs only in western parts of the country. It usually grows into thorny shrubs or small trees up to 4–5 m high. Fruits are spherical or oval, dark red, shiny, 10 mm long, containing usually 2 nutlets, each with furrows (Gostyńska-Jakuszewska 1978, 1979; Zaraś-Januszkiewicz 2002).

The midland hawthorn has considerable ornamental qualities. It flowers very abundantly in May and June. It is also a valuable medicinal plant. Its flowers and fruits are used to produce medicines affecting the heart and nerves and lowering blood pressure.

This species is valuable as a source of generatively propagated rootstocks for hawthorn cultivars. Its cultivars 'Double Pink', 'Paul's Scarlet', 'Punicea' and 'Rubra Plena' produce clusters of double pink to red flowers, while 'Candidoplena', 'Gireoudii', 'Plena', 'Double White', of pink or white flowers (Bärtels 1982, Bugała 1991, Seneta and Dolatowski 2000). These cultivars are propagated by grafting while for the production of rootstocks generative propagation from seeds is used.

Seeds of hawthorns belong to the *orthodox* category, because they can be dried to low moisture content (9–13%) without any considerable loss of viability.

The aim of this study was to determine:

- thermal conditions for the seed dormancy breaking by stratification of nutlets or by their chemical scarification combined with stratification,
- the influence of storage of nutlets dried after harvest or after stratification on seedling emergence rate,
- the effectiveness of controlled conditions applied for seed germination and seedling emergence after the pretreatment of previously stored nutlets.

Methods

Fully ripe fruits of the midland hawthorn (*Crataegus laevigata*) were collected in two locatities (Table 1) in October.

After extracting the nutlets from fruits, the viability of seeds was examined by the cutting test and their moisture content was determined by the oven method (105°C, 24 hours). The dried nutlets were stored at –3°C in sealed containers. The seeds were subjected to warm-followed-by-cold stratification in a moist medium (sand and peat volume ratio 1:1, pH 3.5–4.5) or by scarification in concentrated sulphuric acid followed by soaking in water for 24 hrs; all this in three replications of 50 seeds each.

Afterwards, the seeds were kept at 3~15°C or 3~20°C (16+8 hrs) and subjected separated to germination and seedling emergence tests in the same medium as that used for stratification, lasting 10 weeks. Afterwards the seeds were examined by the cutting test and the results were related to full seeds only (Fig. 1).

In one experimental variant (Fig. 3, experiment 6) the unscarified but stratified nutlets were dried to the moisture content (m.c.) of 13% and stored for one year at –3°C.

In the next variant (Fig. 3, experiment 7), after scarification for 2 or 3 hrs and warm-followed by-cold stratification, the nutlets were dried to the m.c. of 10–11% and stored for one year at –3°C. In experiment 8 the warm phase of stratification was abandoned.

In all experiments the pretreated, dried and stored nutlets were subjected to emergence tests at 3~20°C (16+8 hrs).

Stratification of nutlets was conducted at constant or cyclically alternating temperature (16+8 hrs or 24+24 hrs). Drying of nutlets was performed at first at 3°C for 6 days and afterwards was followed at room temperature for 1 hr in a forced, weak air stream.

Results

Seed germination and seedling emergence from nutlets pretreated by stratification alone

Breaking of dormancy of midland hawthorn seeds is influenced by temperature and duration of both the warm and the cold phase of stratification. Best results of germination and seedling emergence were achieved after warm-followed-by cold stratification with the warm phase at the cyclically alternating temperature of 20~30°C (16+8 hrs) lasting 20 weeks, followed by the cold phase at 3°C lasting 16 weeks. For seedling emergence the temperature of 3~15°C (16+8 hrs) ensured results (94%) and 3~20°C (87%). (Tables 2, 2a, Fig. 4).

In further experiments only one germination temperature at 3~20°C (16+8 hrs) was used, durind germination and seedling emergence test.

Table 1. *Crataegus laevigata*: characteristics of seed lots

Seed lot no.	Origin	Year of harvest	Moisture content of dried nutlets	Storage of nutlets at –3°C		Seed viability*
				time	moisture content	
			%	months	%	%
1	Dobrapomoc, Wielkopolska	2000	8.8	10	9.7	70.0
2				0	8.8	80.0
3a	Sudeten Mts.	2001	13.5	8	13.5	70.0
3b				20	13.8	72.0

*determined by cutting test

In experiment 2, with the warm phase of stratification at 25°C or 20~30°C (16+8 hrs) lasting 12 weeks and the cold phase at 3°C lasting 18–19 weeks, seed germination and seedling emergence did not exceed 46% and 49% respectively. These results confirm the necessity of a longer-lasting warm stratification phase, as in experiment 1 (Table 3).

In experiment 3 the highest level of seedling emergence at 3~20°C (16+8 hrs) was achieved after stratification with the warm phase at 20~30°C (16+8 hrs) or 25°C for 16 weeks followed by a cold phase at 3°C for 16-weeks i.e. 75% and 72% respectively. Higher temperatures of stratification (27.5° or 30°C) were less effective (Tables 4, 4a).

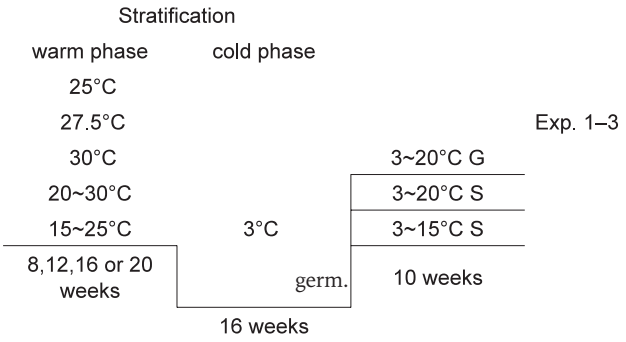

Fig. 1. Pretreatment by stratification (of initially dry-stored nutlets), followed by a germination test or a seedling emergence tests. Experiments 1–3

germ. = initiation of germination (2–4%), G = germination test, S = seedling emergence test

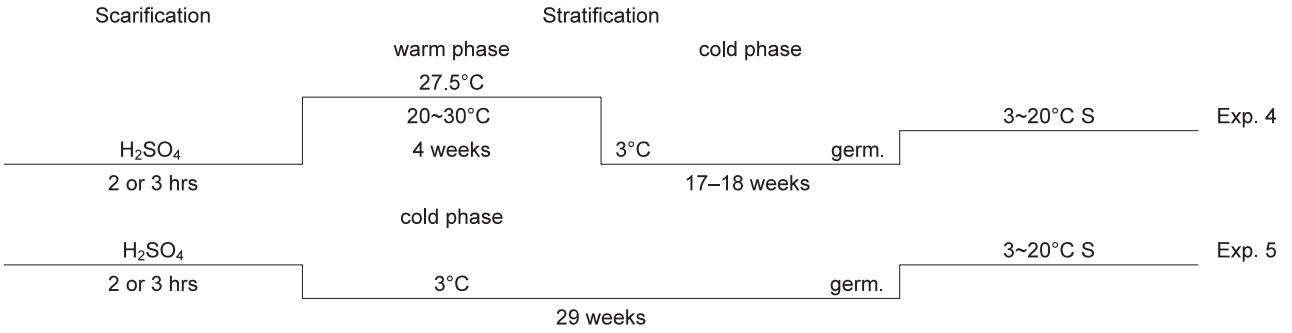

Fig. 2. Pretreatment by scarification and stratification (of initially dry-stored nutlets), followed by seedling emergence test. Experiments 4–5

germ. = initiation of germination (2–4%); S = seedling emergence test

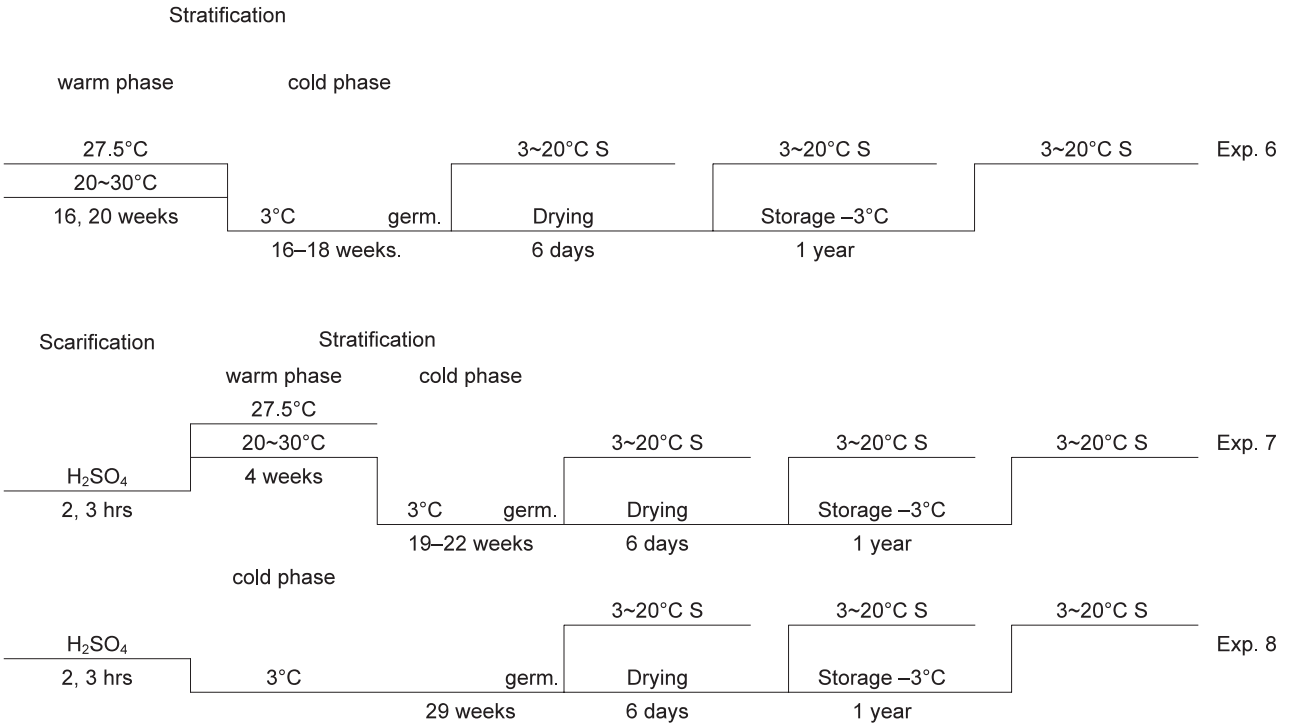

Fig. 3. Pretreatment by stratification or by scarification and stratification (of initially dry-stored nutlets), followed by drying, storage and seedling emergence tests. Experiment 6–8

germ. = initiation of germination (2–4%); S = seedling emergence test

Table 2. *Crataegus laevigata*. Seed germination and seedling emergence (%) in different thermal regimes of the warm-followed-by-cold stratification. Results related to full seeds only. Experiment 1, seed lot 1

Stratification			Germination	Seedling emergence	
warm phase		cold phase 3°C	3~20°C	3~15°C	3~20°C
°C	weeks	weeks	%	%	%
25°	8	16	46	n.d	47
	12		73	69	74
	16		73	80	80
	20		80	80	85
	Mean		**68.0**	**76.3**	**71.5**
20~30° (16+8 hrs)	8	16	36	n.d	43
	12		64	74	65
	16		83	76	78
	20		85	94	87
	Mean		**67.0**	**81.3**	**68.2**
15~25° (16+8 hrs)	8	16	15	n.d	14
	12		40	n.d	30
	16		50	52	57
	20		64	68	67
	Mean		**42.2**	**60.0**	**42.0**

n.d. = no data

Table 2a. *Crataegus laevigata*. Analysis of variance. Experiment 1

Source	DF	SS	F-test	P
Temperature of warm stratification A	2	2834.3130	104.6053	<0.001
Duration of warm stratification B	2	1553.0094	57.3165	<0.001
A*B	4	226.2537	4.1751	<0.0070
Seedling emergence temperature C	1	67.7581	5.0015	<0.0316
A*C	2	133.0897	4.9119	<0.0130
B*C	2	104.3736	3.8521	<0.0305
A*B*C	4	217.5956	4.0154	<0.0086
error	36	487.7156		

Table 3. *Crataegus laevigata*. Seed germination and seedling emergence (%) after stratification at 25°/3° or 20~30°/3°C (16+8 hrs) with the warm phase lasting 12 weeks and the cold phase lasting 18–19 weeks. Results related to full seeds only. Experiment 2, seed lot 2

Stratification			Germination	Seedling emergence
warm phase		cold phase 3°C	3~20°C	3~20°C
°C	weeks	weeks	%	%
25°	12	18	46	n.d.
20~30°		19	40	49

n.d. = no data

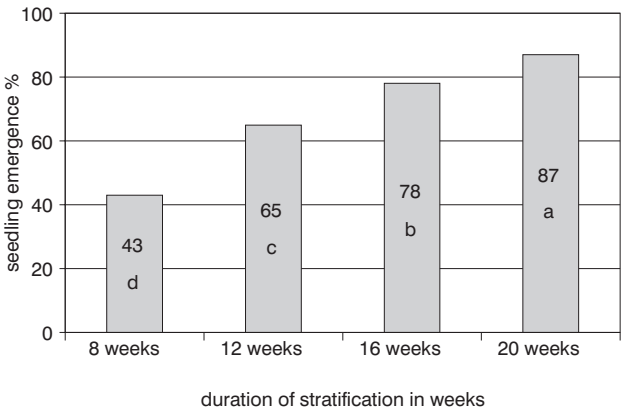

Fig. 4. *Crataegus laevigata* L. Seedling emergence at 3~20°C (16+8 hrs) after warm-followed-by-cold stratification with the warm phase at 20~30°/3°C (16+8 hrs) for 8, 12, 16, 20 weeks and the cold phase for 16 weeks (Tukey test, p≤0,05). Results related to full seeds only. Experiment 1

Table 4. *Crataegus laevigata*. Seedling emergence (%) after warm-followed-by-cold stratification at 25°/3°, 27.5°/3°, 30°/3° or 20~30°/3° C (24+24 hrs) with both the warm and cold phase lasting 16 weeks. Seeds not dried after stratification. Results related to full seeds only. Experiment 3, seed lot 3a

Stratification		Seedling emergence 3~20°C
warm phase	cold phase	
°C	°C	%
25°	3°	72 a
27.5°		66 b
30°		60 c
20~30°		75 a
Mean		**68.2**

Table 4a. *Crataegus laevigata*. Analysis of variance. Experiment 3, seed lot 3 a

Source	DF	SS	F-test	P
Temperature of warm stratification	3	482.79144	11.7940	<0.0001

Seedling emergence after chemical scarification and warm-followed-by-cold stratification

In experiment 4 the nutlets were scarified in concentrated sulphuric acid for 2 or 3 hrs, this followed by warm stratification at 27,5°C for 4 weeks and cold stratification at 3°C for 17–18 weeks. After the scarification and stratification, seedling emergence at 3~20°C (16+8 hrs) achieved high levels, i.e. 80% and 77% for both durations respectively (Tables 5, 5a).

In experiment 5, after scarification of nutlets followed by stratification seedling emergence at 3~20°C (16+8 hrs) achieved the highest levels of 84–87% after both: 2 and after 3 hrs of scarification. After scarification and cold stratification without the warm stratification phase, seedling emergence decreased to 53.5% on average (Tables 6, 6a).

Table 5. *Crataegus laevigata*. Seedling emergence (%) at 3~20°C (16+8 hrs) after scarification and warm-followed-by – cold stratification. Results related to full seeds only. Experiment 4, seed lot 3a

Scarification	Stratification		Seedling emergence 3~20°C
	warm phase 27.5°C	cold phase 3°C	
hrs	weeks	weeks	(%)
2	4	17	80
3		18	77
	Mean		**78.5**

Table 5a. *Crataegus laevigata*. Analysis of variance. Experiment 4, seed lot 3a

Source	DF	SS	F-test	P
Duration of scarification	1	56.3636	4.9677	0.0564

Table 6. *Crataegus laevigata*. Seedling emergence (%) at 3~20°C (16+8 hrs) after scarification and by warm-followed-by-cold stratification. Results related to full seeds only. Experiment 5, seed lot 3b

Scarification	Stratification		Seedling emergence 3~20°C
	warm phase 4 weeks	cold phase 3°C	
hrs	°C	weeks	%
2	n.a	29	49
3			58
	Mean		**53.5**
2	27.5°	21	85
3		22	87
	Mean		**86.0**
2	20~30°	19	84
3			85
	Mean		**84.5**

n.a. = not applied

Seedling emergence after storage of nutlets non-pretreated or pretreated by chemical scarification preceding warm-followed-by-cold stratification

In experiment 3 the nutlets were dried to a m.c. of 10–11% after warm-followed-by-cold stratification at 25°/3°C; 27.5°/3°C or 30°/3° (16+16 weeks) or after warm-followed-by-cold stratification at 20~30°C/3°C (cycle 24+24 hrs, 16+16 weeks). Drying after stratification resulted in a considerable decrease in seedling emergence at 3~20°C from 68% for undried seeds to 49% for the dried seeds (Table 7). This demonstrates the negative effect of drying of already pretreated midland hawthorn seeds. Higher levels of seedling emergence were ensured when a lower temperature (25° or 20~30°C) was applied in the warm phase of stratification of nutlets.

Table 7. *Crataegus laevigata*. Effect on seedling emergence of drying of nutlets after warm-followed-by-cold stratification. Results related to full seeds only. Experiment 3, seed lot 3a

Stratification		Moisture content of nutlets dried after stratification	Seedling emergence 3~20°C (%)	
warm phase 16 weeks	cold phase 16 weeks		undried nutlets	dried nutlets
°C	°C	%	%	%
25°	3°	10.2	72	57
27.5°		10.1	66	54
30°		10.8	60	32
20~30°		10.5	75	53
	Mean		**68.2**	**49.0**

Table 8. *Crataegus laevigata*. Seedling emergence at 3~20°C (16+8 hrs) after warm- followed-by-cold stratification of seeds undried or dried, or dried and stored at –3°C. Results related to full seeds only. Experiment 6, seed lot 3b

Stratification			Moisture content of nutlets		Seedling emergence at 3~20°C		
warm phase		cold phase	after stratification	after stratification and storage	undried nutlets	dried nutlets	dried and stored nutlets
°C	weeks	weeks	%	%	%	%	%
27.5°	16	17	11.8	12.0	83	75	72
	20	16	10.3	11.9	73	62	62
	Mean				**78.0**	**68.5**	**67.0**
20~30° (24+24 hrs.)	16	17	11.9	13.0	96	89	83
	20	16	13.0	13,5	98	91	93
	Mean				**97.0**	**90.0**	**88.0**

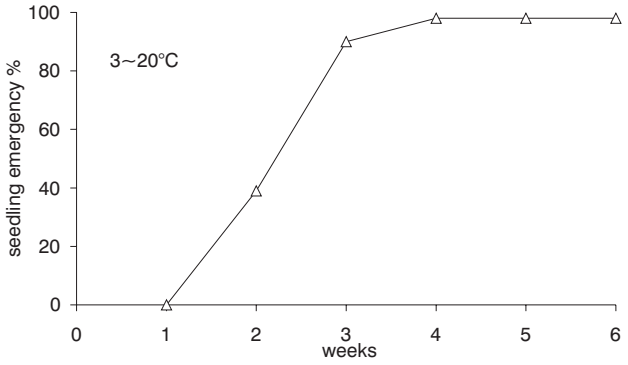

Fig. 5. *Crataegus laevigata* L. The course of seedling emergence after warm-followed-by-cold stratification with the warm phase at cyclically alternating temperature 20~30°C (24+24 hrs) for 20 weeks and the cold phase at 3°C for 16 weeks. Experiment 6

In the season of 2002/2003 further investigations were carried out with nutlets dried after collection in 2001 to a m.c. of 13% and stored afterwards at –3°C in sealed containers for 20 months (experiment 6). After the initial storage, warm-followed-by-cold stratification was conducted at 27.5°/3°C or 20~30°C/3°C (16 or 20 weeks of warm and 16–17 weeks of cold) and the nutlets were dried again to a m.c. of 10–13%, to be stored for one year at –3°C. Drying of nutlets after stratification resulted in a decrease in seedling emergence at 3~20°C (mean for undried seeds 97%, for dried seeds 90%, for dried and stored seeds 88%) when the warm phase of stratification was run at 20~30°C (Table 8, Fig. 5).

In experiments 7 and 8 the nutlets were scarified for 2 or 3 hrs with concentrated sulphuric acid and next subjected to warm-followed-by-cold stratification with the warm phase at 27.5°C or 20~30°C (24+24 hrs) for 4 weeks, and the cold phase at 3°C continued until the first radicles started to appear (16–17 weeks). After the treatments, the nutlets were dried to a m.c. of 11–13% and stored for one year at –3°C. A relatively high level of seedling emergence (78–79%) was obtained under such conditions. However, drying of nutlets reduced (by 4–8%) seedling emergence as compared to the undried seeds (Table 9).

Scarification (Table 9, Fig. 6, B, C, D) contributed to some reduction of seedling emergence compared with the unscarified nutlets (A). This reduction was

Table 9. *Crataegus laevigata*. Seedling emergence at 3~20°C (16+8 hrs) of undried, dried or dried and stored nutles at –3°C after scarification and cold only or warm- followed-by-cold stratification. Results related to full seeds only. Experiments 7 and 8, seed lot 3b

Scarification	Stratification		Moisture content of nutlets		Seedling emergence 3~20°C		
	warm phase 4 weeks	cold phase 3°C	after stratification	after stratification and storage	undried nutlets	dried nutlets	dried and stored nutlets
hrs	°C	weeks	%	%	%	%	%
2	n.a.	29	11.5	12.1	49	41	40
3			11.3	11.4	58	47	47
	Mean				**53.5**	**44.0**	**43.5**
2	27.5°	21	13.1	13.6	85	80	79
3		22	12.3	12.4	87	79	78
	Mean				**86.0**	**79.5**	**78.5**
2	20~30°	19	11.8	12.1	84	79	78
3			11.6	11.2	85	77	78
	Mean				**84.5**	**78.0**	**78.0**

n.a. = not applied

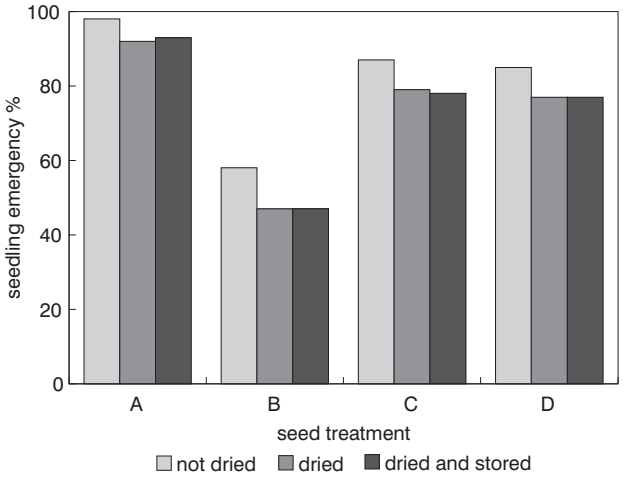

Fig. 6. *Crataegus laevigata*. Seedling emergence at 3~20°C (16+8 hrs) after warm-followed-by-cold stratification at 20~30°C (24+24 hrs/3°C, 20+16 weeks) (**A**); scarification for 3 hrs and stratification at 3°C (**B**); scarification for 3 hrs and stratification at 27.5°/3°C (4+22 weeks) (**C**); scarification for 3 hrs and stratification at 20~30°C/3°C (4+19 weeks) (**D**). Experiments 7 and 8

lesser when the scarification preceded warm-followed-by-cold stratification rather than cold stratification. Storage of nutlets lasting one year did not reduce seedling emergence when compared with dried but non-stored seeds.

Discussion

There exists some published information on seed pretreatment in the common hawthorn (*C. monogyna*) and some American species of hawthorns (Buszewicz and Holmes 1955, Brinkman 1974; Crocker 1948; Gordon and Rowe 1982; St-John-S 1982, Dirr and Heuser 1987; Nyholm 1975, ISTA 1999, Young and Young 1992, Morgenson 2000, Piotto 2002). Those findings were used for the planning of this study. However, for the midland hawthorn no data were available about seed dormancy breaking.

The main assumption of this study was that the basic method of seed dormancy breaking in the midland hawthorn is seed stratification, conducted in suitable, controlled temperatures. This assumption derived from Tyszkiewicz's (1949) observation that hawthorn nutlets, sown in spring in a nursery, germinated in the next spring. Another assumption was that shortening of stratification would be favourable.

So far, cyclically alternating temperature has not been used for seed dormancy breaking in hawthorns. In this study such thermal conditions proved to be very useful. Also the frequency of changes in temperature at this stage of stratification were important. In practice it is advisable to use the cycle 24+24 hrs (Tables 2 and 4).

During the period of appearance of first radicles at the cold stage of stratification (3°C), most of seeds in the whole or broken stones were still dormant, so they required prolonged stratification at the low temperature. The compared alternating temperature cycles, both 3~15°C and 3~20°C (16+8 hrs), were equally effective (Table 2). The cyclically alternating temperature resembles the natural thermal conditions in the soil at the depth of sowing the nutlets in March and April, when ground frost is possible at dawn, while temperature during the day can exceed 20°C. Such alternating temperature was first applied in research on seed dormancy breaking *Berberis Thunbergii* in 1923 by Morinaga (1926), also for seed germination tests and seedling emergence tests. In Poland the cyclically alternating temperature was first used successfully by Suszka (1967) during a study of germination and seedling emergence from stratified stones of mazzard cherry (*Prunus avium* L.).

In this study, nutlets of the midland hawthorn were subjected to warm-followed-by-cold stratification with the warm phase at 25°, 27.5° and 30°C or at cyclically alternating temperature 15~25° or 20~30°C (16+8 hrs and 24+24 hrs) for 8, 12, 16 and 20 weeks, followed by the cold phase at 3°C for 16–18 weeks (until first radicles start to appear).

The results show that dormancy of seeds of the midland hawthorn can be optimally overcome by a long-lasting stratification in a moist medium in the thermal regime 20~30°C/3°C, namely 16–20 weeks at 20~30°C (16+8 hrs or 24+24 hrs) followed by 16-18 weeks at 3°C, i.e. to the time when the first radicles start to appear.

The duration of warm stratification could be greatly shortened (to 4 weeks, i.e. by 10 or more weeks) if the hard endocarp was scarified with concentrated sulphuric acid for 2–3 hrs. The effectiveness of this method was only slightly lower than of warm-followed-by-cold stratification without preceding scarification.

The process of dormancy release after stratification or scarification linked with stratification could be suspended by means of partial dehydration of the nutlets. This can be useful in practice if the sowing date must be postponed, for example because of unfavourable weather conditions.

Seedling emergence rate in the laboratory was always lower for hawthorn seeds dried after stratification to the m.c. of 10–13%, as compared with undried seeds, but it was still high, about 80% (Table 8). Such partly dried seeds were stored at the temperature of –3°C in tightly closed containers for one year and their emergence rate was the same as in the case of non-stored seeds (Table 9). This enables successful storage of pretreated hawthorn seeds.

Conclusions

1. Seed dormancy in the midland hawthorn can be optimally overcome by stratification in a moist medium (sand and peat, volume ratio 1:1) in the following thermal regime: warm-followed-by-cold stratification with the warm phase at cyclically alternating temperature 20~30° (24+24 hrs) for 20 weeks followed by the cold phase at 3°C, i.e. until first radicles start to appear. Such pretreated seeds germinate vigorously (in 3–5 weeks) and at a high percentage at 3~20°C (16+8 hrs). After sowing of such stratified nutlets the seedlings emerge at 3~15°C (16+8 hrs) or 3~20°C (16+8 hrs) in 4-6 weeks.
2. Scarification of nutlets in concentrated sulphuric acid for 2 or 3 hrs, followed by stratification for 4 weeks at 3°C, has a negative effect on seedling emergence at 3~20°C and does not shorten the duration of the stratification period. If scarification of nutlets was followed by warm stratification for 4 weeks at 27.5°C or at cyclically alternating temperature 20~30°C (24+24 hrs), and afterwards by stratification at 3°C, then seedling emergence reached a high level (mean 87%), and duration of the warm phase of stratification was shortened by more than 10 weeks.
3. The obtained results suggest that after stratification nutlets of the midland hawthorn can be dried to a m.c. of 10-13%. This reduced seedling emergence only by 4-8%, in contrast to seeds with a m.c. of 10-11%, where the decline of seedling emergence was much stronger.
4. Storage for one year at –3°C of nutlets dried to a m.c. of 10–13% after stratification or scarification combined with stratification does not reduce their seedling emergence (mean 71%).

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